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(71) Applicant *(for all designated States except US)*: THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : ABRAHAM, Irene [US/US]; 1805 Waite, Kalamazoo, MI 49008 (US). ARISTOFF, Paul, A. [US/US]; 1650 Brookmoor Lane, Portage, MI 49002 (US). SKULNICK, Harvey, I. [US/US]; 1745 Old Deer Run, Kalamazoo, MI 49009 (US).

(74) Agent: WOOTTON, Thomas, A.; The Upjohn Company, 301 Henrietta Street, Kalamazoo, MI 49001 (US).

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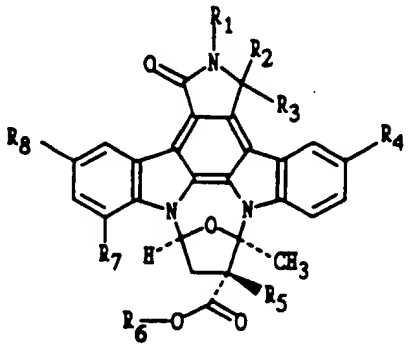
(54) Title: PROTEIN KINASE INHIBITORS AND RELATED COMPOUNDS COMBINED WITH TAXOL



(I)

(57) Abstract

This invention describes both known and novel compounds, some of which are protein kinase inhibitors, that may be combined with taxol type compounds. The combination of disclosed compounds plus taxol type compounds exhibits powerful synergistic effects and the combinations are useful in the treatment of cancer. The novel compounds and their synthesis are described. A compound of formula (I), above, is described wherein $R_1$-$R_8$ represent various substituents.

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# PROTEIN KINASE INHIBITORS AND RELATED COMPOUNDS COMBINED WITH TAXOL

## FIELD OF THE INVENTION

This invention describes the use of compounds that are used in combination with taxol to control cancerous growths and tumors. Protein kinase inhibitors and related compounds are combined with taxol and taxol related compounds and the combination of compounds exhibits powerful potentiating effects when used to treat cancer. Many of the compounds are protein kinase inhibitors, other compounds achieve similar effects but are not necessarily protein kinase inhibitors.

## BACKGROUND OF THE INVENTION

Taxol was first isolated from the bark of the western yew, *Taxus brevifolia*, and identified as an antitumor agent in 1971 by Wani, et al. Recently, phase II clinical trials with taxol have shown it to be one of the most exciting chemotherapeutics available. Taxol has proven effective in drug-refractory ovarian cancer (McGuire, et al., 1989), and has shown a 56% objective response rate in metastatic breast cancer (Holmes, et al., 1991). In addition, there is reason for hope that taxol may be effective in many other types of cancers.

The development of taxol, however, has faced many obstacles. Taxol's poor solubility required that it be administered in the vehicle Cremophor EL (polyethylated castor oil), which led to a high incidence of hypersensitivity reactions. It is not clear whether these reactions are caused by the vehicle or the drug, but it was found that using longer drug infusions (Weiss, et al., 1990) and anti-allergic regimens (Rowinsky, et al., 1990) reduced the incidence of such reactions. In addition, there are inherent problems in producing sufficient quantities of taxol. Extraction from the bark of the extremely slow growing western yew using present methods cannot meet the demand for taxol. Cultivation of the western yew may take years to establish, synthesis of the complex taxol molecule will be difficult and/or very expensive. Alternative sources of taxol or a taxol substitute or a taxol additive would therefore be highly desirable.

Taxol has been shown previously to be toxic to tumor and leukemia cells inoculated in mice, including L-1210, P388 and P-1534 leukemia cells and Walker WM-256 carcinosarcoma, sarcoma 180 and Lewis lung tumor cells (Wani et al., 1971). It has also been shown to be toxic to cultured human HeLa cells (Schiff et al., 1979 ) and CHO (Chinese hamster ovary) cells (Cabral et al., 1981). This evidence of toxicity to rodent and human tumor cells *in vitro* and to tumor bearing mice *in vivo* predicted that taxol would be an active chemotherapeutic agent and led to clinical trials in human cancer patients. These clinical trials showed efficacy of taxol in treating ovarian cancer (McGuire et al., 1989). Taxotere is a taxol type compound that has also been shown to have powerful antitumor activity. Bissery et. al, Cancer Research 51, 4845-4852, Sept. 15, 1991.

Since taxol is now known to be an effective chemotherapeutic agent, a co-treatment that increases the toxicity of taxol on cancer or transformed cells, such as CHO cells, would be likely to increase the chemotherapeutic effect of taxol in cancer patients or to allow smaller doses of taxol to be administered. Quantities of taxol available are extremely limited. Compounds that increase the efficacy of taxol, thereby allowing smaller amounts to be used with equal effectiveness, will enable more patients to be treated with taxol. This should also reduce the hypersensitivity and non-therapeutic toxic reactions seen clinically with taxol, as both less taxol and the less of the vehicle needed to deliver taxol will need to be administered.

Our finding is that when the compounds of this invention are combined with taxol or taxol related compounds the mixture of compounds has a potentiating effect that, surprisingly produces tumor cell toxicity at lower doses than taxol alone. These findings, and other studies, suggest that the compounds will be effective in synergizing with taxol in killing tumor cells in human cancer patients. The findings also suggest results may be seen with taxol related compounds such as taxotere and related taxol analogues.

## INFORMATION DISCLOSURE

Many of the compounds of this invention are related to the physiologically active substance K-252. The following patents disclose some of these compounds. U.S. 4,877,776 issued October 31, 1989. U.S. 4,923,986 issued May 8, 1990. W.O 8807-045-A published September 22, 1988.

The following Japanese patent applications also disclose related compounds: J63 295-588-A, J63 295-589-A, J62 155-284-A, and J62 155-285-A disclose staurosporine related compounds.

## SUMMARY OF THE INVENTION

This invention is in two parts. Known compounds are listed in part I, they are claimed for the method of using the compounds as described herein. The known compounds are also claimed as compounds combined in a composition with taxol type compounds. The new compounds are in part II. The new compounds are claimed as compounds, for their method of use and in a composition.

I. The known compounds.

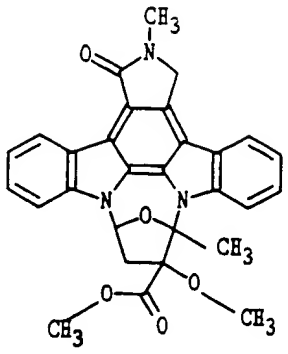
A. Indolocarbazole Type Compounds

1) The "First Known Derivatives of K-252." The "First Known Derivatives of K-252" are all of the compounds disclosed in U.S. patent 4, 877,776. U.S. patent 4, 877,776 incorporated herein by reference.

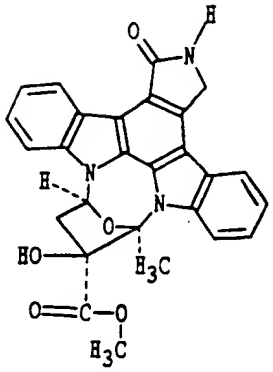
2) The "Second Known Derivatives of K-252." The "Second Known Derivatives of K-252" are all of the compounds disclosed in U.S. patent 4,923,986. U.S. patent 4,923,986 incorporated herein by reference.

3) The specific compounds below are more preferred.

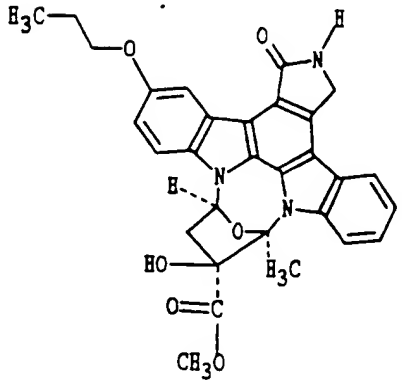
3(a) KT5823



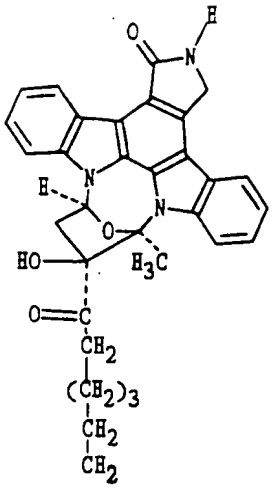
3(b) K-252A



3(c) KT5926



3(d) KT5720



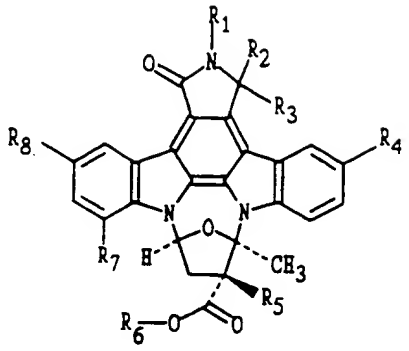
3(e) Staurosporine

B. Non-Indolecarbazole Type Compounds

1) Adriamycin
2) Amiloride
3) Calphostin
4) Chlorpromazine
5) The compound known as "HA-1004"
6) Indomethacin
7) Okadaic acid
8) Phenazocine
9) Polymyxin B
10) 2-aminopurine
11) 6-dimethyl-aminopurine
12) Sphingosine
13) Tamoxifen
14) Compounds related to tamoxifen such as triphenylethylene antiestrogens
15) Trifluoperazine
16) Verapamil
17) 3-isobutyl-1-methyl-xanthine
18) 8-Cl-cAMP

II. The new compounds.

A compound of FORMULA I, below,



FORMULA I

wherein,

$R_1$ is -H, -($C_1$-$C_4$ alkyl), -C(O)-($C_1$-$C_4$ alkyl), -$NH_2$, -C(O)-$NH_2$, -$CH_2CH_2$-N($R_{1-1}$)$_2$,

wherein $R_{1-1}$ is -H or -($C_1$-$C_4$) alkyl,

$R_2$ is -H, or $R_2$ and $R_3$ taken together are (O),

$R_3$ is -H, -OH or $R_2$ and $R_3$ taken together are (O),

$R_4$ is -H, -OH, -$NH_2$, or -O-($C_1$-$C_4$ alkyl),

$R_5$ is -OH, -O-($C_1$-$C_4$ alkyl), or -O-C(O)-($C_1$-$C_4$ alkyl),

$R_6$ is -($C_6$-$C_{12}$ alkyl), -($C_3$-$C_{10}$ cycloalkyl), -$(CH_2)_nCH_2N(R_{6-1})_2$,

wherein $R_{6-1}$ is -H, or -($C_1$-$C_4$ alkyl),

$R_7$ is -H, or -$NH_2$,

$R_8$ is -Cl, -Br, -H, -$CH_3$, -$CH_2$OH, -OH; -O-($C_1$-$C_4$ alkyl), -N($R_{8-1}$)$_2$, or -NHC(O)-NH($R_{8-1}$),

wherein $R_{8-1}$ is -H or -($C_1$-$C_4$ alkyl)

wherein n is 0-5

with the proviso that:

a) when $R_2$ or $R_3$ is -OH then $R_1$ is H,

b) when $R_1$, $R_2$, $R_3$, $R_4$, and $R_7$ all equal H and $R_5$ is OH then $R_6$ does NOT equal -$(CH_2)_5CH_3$,

c) when $R_1$, $R_4$, and $R_7$ all equal H, and $R_2$ combined with $R_3$ is (O), and $R_5$ is OH , then $R_6$ does NOT equal -$(CH_2)_5CH_3$.

d) when $R_4$ is -OH, -$NH_2$, or -O($C_1$-$C_4$ alkyl), then $R_4$ and $R_8$ are the same.

A pharmaceutical composition consisting of a pharmaceutically acceptable carrier and an effective amount of FORMULA I. A pharmaceutical composition consisting of a

pharmaceutically acceptable carrier and an effective amount of the compound of FORMULA I in conjunction with an appropriate dose of taxol or taxol related compounds. A method of controlling cancerous growths in mammals which comprises administering a therapeutic or prophylactic dosage of any of the three following groups of compounds in conjunction with an appropriate dose of taxol or taxol related compounds. 1) a compound of FORMULA I, 2) any one of the compounds described in the specification as "Indolecarbazole Type Compounds.", 3) any one of the compounds described in the specification as "Non-Indolecarbazole Type Compounds."

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Isobologram showing potentiating effect of the combination of Taxol plus KT5823. The isobologram shows the effectiveness of a combination of 2 drugs for the killing of wild type, 10001a CHO, cells. The data line is the solid line with open circle or triangle data points. The data line shows the combination of doses which gives an $LD_{50}$ for the cells. The diagonal dashed line shows the predicted concentrations of drugs if their combination only had an additive effect. If any data points were above the dashed line that would indicate the combination of compounds had antagonistic effects. Data points below the line indicate the compounds have potentiating or synergistic effects.

Figure 2. Isobologram showing potentiating effect of the combination of taxol plus KT5926.

Figure 3. Isobologram showing potentiating effect of the combination of taxol plus KT5720.

Figure 4. Isobologram showing NO potentiating effect from the combination of taxol plus H-9. This isobologram shows the predicted effect of a "control" substance that does NOT act in a potentiating or synergistic manner.

Figure 5. Isobologram showing potentiating effect of the combination of taxol plus K252a.

Figure 6. Isobologram showing potentiating effect of the combination of taxol plus tamoxifen.

Figure 7. Isobologram showing potentiating effect of the combination of taxol plus 2-aminopurine.

Figure 8. Isobologram showing potentiating effect of the combination of taxol plus 6-dimethylaminopurine.

Figure 9. Isobologram showing potentiating effect of the combination of taxol plus chlorpromazine.

Figure 10. Isobologram showing potentiating effect of the combination of taxol plus 3-isobutyl-1-methyl-xanthine.

Figure 11. Isobologram showing potentiating effect of the combination of taxol plus 8-Cl-cAMP.

Figure 12. Isobologram showing potentiating effect of the combination of taxol plus Example A-1.

Figure 13. Isobologram showing potentiating effect of the combination of taxol plus Example B-1.

Figure 14. Isobologram showing potentiating effect of the combination of taxol plus Example B-2.

Figure 15. Isobologram showing potentiating effect of the combination of taxol plus Example B-3.

Figure 16. Isobologram showing potentiating effect of the combination of taxol plus Example B-4.

Figure 17. Isobologram showing potentiating effect of the combination of taxol plus Example B-5.

Figure 18. Isobologram showing potentiating effect of the combination of taxol plus Example B-6.

Figure 19. Isobologram showing potentiating effect of the combination of taxol plus Example B-7.

Figure 20. Isobologram showing potentiating effect of the combination of taxol plus Example B-8.

Figure 21. Isobologram showing potentiating effect of the combination of taxol plus Example B-9.

Figure 22. Effect of KT5720 and taxol on the growth of MX-1 tumors.

Figure 23. Table of data showing toxicity of several of the drugs both individually and in combination with taxol on non-tumored mice. (In Vivo Effects)

## DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are of two types. The first type are known compounds described here for their usefulness when combined with taxol type compounds and used to treat cancer. The second type of compounds are novel compounds described here for the first time. These novel compounds are also useful when combined with taxol type compounds and used for the treatment of cancer.

### I. Known Compounds

I. The known compounds, and the source of those compounds, are listed below, and described by name and by reference to the labeled structures.

A) Indolocarbazole Type Compounds

1) The "First Known Derivatives of K-252." The "First Known Derivatives

of K-252" are all of the compounds disclosed in U.S. patent 4, 877,776. U.S. patent 4, 877,776 incorporated herein by reference.

2) The "Second Known Derivatives of K-252." The "Second Known Derivatives of K-252" are all of the compounds disclosed in U.S. patent 4,923,986. U.S. patent 4,923,986 incorporated herein by reference.

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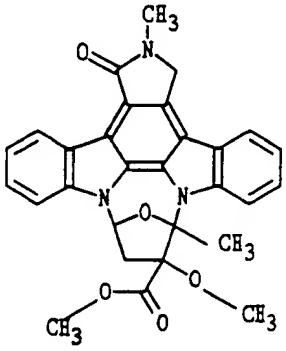
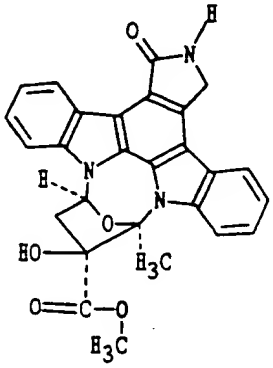
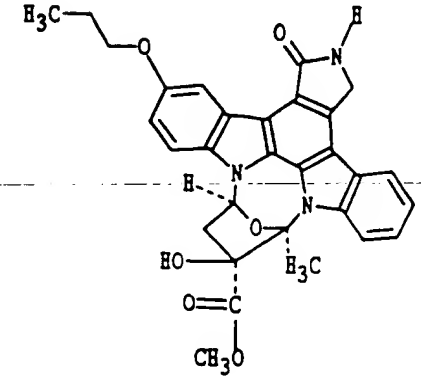


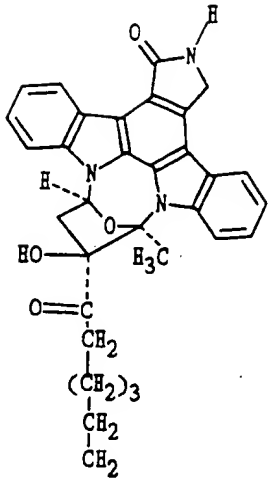
3(b) K-252A



3(c) KT5926



3(d) KT5720



3(e) Staurosporine

B. Non-Indolecarbazole Type Compounds

1) Adriamycin[S]
2) Amiloride[S]
3) Calphostin[S]
4) Chlorpromazine[S]
5) The compound known as "HA-1004"
6) Indomethacin[S]
7) Okadaic acid
8) Phenazocine[S]
9) Polymyxin B[S]
10) 2-aminopurine[S]
11) 6-dimethyl-aminopurine[S]
12) Sphingosine[S]
13) Tamoxifen
14) Compounds related to tamoxifen such as triphenylethylene antiestrogens
15) Trifluoperazine[S]
16) Verapamil[S]
17) 3-isobutyl-1-methyl-xanthine[S]
18) 8-Cl-cAMP

Compounds marked with a superscript [S] are available from Sigma Chemical Company. Taxol and taxotere can be obtained from The National Cancer Institute.

The clinical pharmacology of taxol is reviewed by Eric K. Rowinsky and Ross C. Donehower, The Clinical Pharmacology and Use of Antimicrotubule Agents in Cancer Chemotherapeutics, Pharmac. Ther., Vol 52, pp 35-84, 1991. Clinical and preclinical studies with taxol are reviewed by William J. Slichenmyer and Daniel D. Von Hoff, Taxol: A New and Effective Anti-cancer Drug, Anti-Cancer Drugs, Vol. 2, pp 519-530, 1991.

Taxol and analogs thereof are the subject of various patents including, for example, U.S. Patent Nos. 4,814,470; 4,857,653; 4,942,184; 4,924,011; 4,924,012; 4,960,790; 5,015,744; 5,157,049; 5,059,699; 5,136,060; 4,876,399 as well as PCT Publication No. WO 92/09589, European Patent Application 90305845.1 (Publication No. A2 0 400 971), 89400935.6 (Publication No. A1 0 366 841) and 90402333.0 (Publication No. 0 414 610 A1), 87401669.4 (A1 0 253 739), and PCT Publication Nos. WO 91/17977, WO 91/17976, WO 91/13066, WO 91/13053.

Rebeccamycin is described in: T. Kaneko and H. Wong, Tetrahedron Letters, Vol. 26, No. 34, pp 4015-4018 (1985).

The compounds known as K252a, K252b, KT5720, KT5823, KT5926, okadaic acid and staurosporine, are available from Kamiya Biomedical Company, Thousand Oaks, California.

The compounds known as "H-7," "H-9" and "HA-1004" (B4-B6) are available from Seikagaku America, Inc., St. Petersburg, Florida

Lavendustin (B8) is available from Gibco BRL.

The compound, Kampferol-7-neonesperidoside, is available from Apin Chemical Co., Abingden, Oxfordshire, United Kingdom.

All of the documents referred to above are incorporated by reference herein.

## II. New Compounds

II. The new compounds of this invention are identified in two ways: by the descriptive name and by reference to structures contained in appropriate charts. In some situations, the proper stereochemistry is also represented in the charts.

In this document the parenthetical term ($C_n$-$C_m$) is inclusive such that a compound of ($C_1$-$C_8$) would include compounds of one to 8 carbons and their isomeric forms. The various carbon moieties are defined as follows: Alkyl refers to an aliphatic hydrocarbon radical and includes branched or unbranched forms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, isopentyl, n-hexyl, isohexyl, n-heptyl, isoheptyl, and n-octyl.

Alkoxy as represented by -O-($C_1$-$C_8$ alkyl) refers to an alkyl radical which is attached to the remainder of the molecule by oxygen and includes branched or unbranched forms such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, t-butoxy, n-pentoxy, isopentoxy, n-hexoxy, isohexoxy, n-heptoxy, isoheptoxy, and n-octoxy.

$(C_3-C_{10})$cycloalkyl refers to a radical of a saturated cyclic hydrocarbon which includes alkyl-substituted cycloalkyl, such as cyclopropyl, 2-methylcyclopropyl, 2,2-dimethylcyclopropyl, 2,3 diethylcyclopropyl, 2-butylcyclopropyl, cyclobutyl, 2-methylcyclobutyl, 3-propylcyclobutyl, cyclopentyl, 2,2-dimethylcyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl. Each of these moieties may be substituted as appropriate.

It will be apparent to those skilled in the art that compounds of this invention may contain chiral centers. The scope of this invention includes all enantiomeric or diastereomeric forms of formula I compounds either in pure form or as mixtures of enantiomers or diastereomers. The therapeutic properties of the compounds may to a greater or lesser degree depend on the stereochemistry of a particular compound.

Both organic and inorganic acids can be employed to form non-toxic pharmaceutically acceptable acid addition salts of the compounds of this invention. Illustrative acids are sulfuric, nitric, phosphoric, hydrochloric, citric, acetic, lactic, tartaric, palmoic, methanesulfonic, ethanedisulfonic, sulfamic, succinic, cyclohexylsulfamic, fumaric, maleic, and benzoic acid. These salts are readily prepared by methods known in the art.

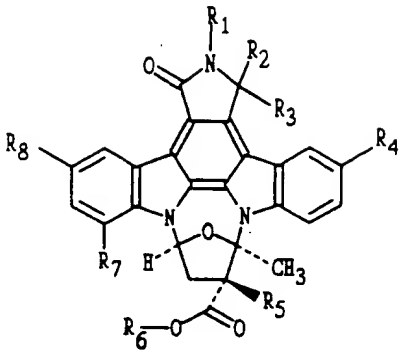
The compounds of this invention can be made in accordance with the processes described in the PREPARATIONS AND EXAMPLES for the preparation of novel compounds and illustrated in the GENERAL REACTIONS and the REACTIONS OF CHART A and CHART B.

In clinical practice the compounds of the present invention will normally be administered by injection, in the form of pharmaceutical preparations comprising the active ingredient either as a free base or as a pharmaceutically acceptable non-toxic, acid addition salt, such as the hydrochloride, lactate, acetate, mesylate, methanesulfonate, or sulfamate salt, in association with a pharmaceutically acceptable carrier. The use and administration to a patient to be treated in the clinic would be readily apparent to a physician or pharmacist of ordinary skill in the art.

In therapeutical treatment the suitable daily doses of the compounds of the invention should fall within the following ranges: Taxol, taxotere and related compounds should be administered from .001 mg/kg to 10 mg/kg, preferably between .05 mg/kg to 5 mg/kg for intravenous administration. The compounds to be combined with taxol should be administered in the same dosage range. The precise dosage will be apparent to an ordinarily skilled physician or pharmacologist taking into account factors such as the age, weight, sex, and medical condition of the patient being treated. Also relevant is the potency of the particular compound and its ability to potentiate the effects of taxol. The potency of the compounds are indicated by the standard tests described below.

The New Compounds:

A compound of FORMULA I, below,



FORMULA I

wherein,

$R_1$ is -H, -($C_1$-$C_4$ alkyl), -C(O)-($C_1$-$C_4$ alkyl), -$NH_2$, -C(O)-$NH_2$, -$CH_2CH_2$-N($R_{1-1}$)$_2$,

wherein $R_{1-1}$ is -H or -($C_1$-$C_4$ alkyl),

$R_2$ is -H, or $R_2$ and $R_3$ taken together are (O),

$R_3$ is -H, -OH or $R_2$ and $R_3$ taken together are (O),

$R_4$ is -H, -OH, -$NH_2$, or -O-($C_1$-$C_4$ alkyl),

$R_5$ is -OH, -O-($C_1$-$C_4$ alkyl), or -O-C(O)-($C_1$-$C_4$ alkyl),

$R_6$ is -($C_6$-$C_{12}$ alkyl), -($C_3$-$C_{10}$ cycloalkyl), -$(CH_2)_nCH_2N(R_{6-1})_2$,

wherein $R_{6-1}$ is -H, or -($C_1$-$C_4$ alkyl),

$R_7$ is -H, or -$NH_2$,

$R_8$ is -Cl, -Br, -H, -$CH_3$, -$CH_2$OH, -OH, -O-($C_1$-$C_4$ alkyl), -N($R_{8-1}$)$_2$, or -NHC(O)-NH($R_{8-1}$),

wherein $R_{8-1}$ is -H or -($C_1$-$C_4$ alkyl)

wherein n is 0-5

with the proviso that:

a) when $R_2$ or $R_3$ is -OH then $R_1$ is H,

b) when $R_1$, $R_2$, $R_3$, $R_4$, and $R_7$ all equal H and $R_5$ is OH then $R_6$ does NOT equal -$(CH_2)_5CH_3$,

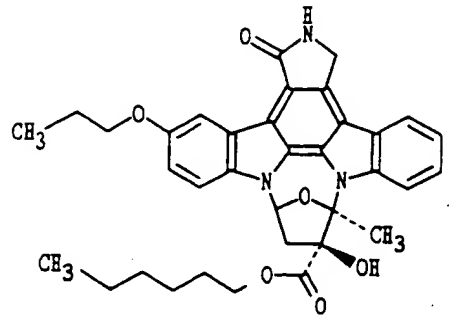
c) when $R_1$, $R_4$, and $R_7$ all equal H, and $R_2$ combined with $R_3$ is (O), and $R_5$ is OH , then $R_6$ does NOT equal -$(CH_2)_5CH_3$.

d) when $R_4$ is -OH, -$NH_2$, or -O-($C_1$-$C_4$ alkyl), then $R_4$ and $R_8$ are the same.

Preferred Compounds

The preferred compounds of this invention are those, referring to the compound of FORMULA I, wherein $R_1$ is H or $CH_3$; $R_2$, $R_3$, and $R_7$ is H; $R_5$ is OH or $OCH_3$;

$R_8$ is -O-($C_1$-$C_4$ alkyl). The following compounds are preferred, Example B-4 and Example A-1, Example A-1 whose structure is shown below.



(Example A-1)

Biological Activity

Since taxol is known to be an effective chemotherapeutic agent, for example in the treatment of ovarian cancer, any co-treatment that increases the toxicity of taxol on cancer cells, such as CHO cells, would be likely to increase the chemotherapeutic effect of taxol in cancer patients or to allow smaller doses of taxol to be administered. The compounds of this invention synergize with taxol to produce tumor cell toxicity at lower doses than taxol alone, this requires the conclusion that the compounds will be effective in synergizing with taxol in killing tumor cells in human cancer patients. Additional studies that evaluate the compounds effects on human breast call MX-1 tumors, described below also support this conclusion.

The compounds of this invention are therefore useful for the same cancers for which taxol has been shown active, including human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia. See, e.g., the clinical pharmacology of taxol is reviewed by Eric K. Rowinsky and Ross C. Donehower, The Clinical Pharmacology and Use of Antimicrotubule Agents in Cancer Chemotherapeutics, Pharmac. Ther., Vol 52, pp 35-84, 1991. Clinical and preclinical studies with taxol are reviewed by William J. Slichenmyer and Daniel D. Von Hoff, Taxol: A New and Effective Anti-cancer Drug, Anti-Cancer Drugs, Vol. 2, pp 519-530, 1991.

Cell lines and growth.

The parental CHO line, 10001a, is a subclone of the CHO line $Pro^{-5}$ (Stanley et al., 1975). The line was maintained in alpha-MEM Earle's Salts supplemented with 2 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum. All cell lines were maintained at 37°C in 5% $CO_2$ in a humidified incubator. Periodically, the cell lines were tested for mycoplasma and always found to be free of infection. Compounds were dissolved in dimethylsulfoxide (DMSO) and then diluted into medium for cell

growth assays.

Drug synergy experiments - "10001a" cell lines.

Cells were treated simultaneously with the experimental compound and taxol in 132 different combinations of doses in 96 well plates. The 96-well plates were incubated for four days. Cell growth was determined by the development of the colorimetric dye 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described by Mosmann, 1983. MTT dissolved in PBS at 2 mg/ml was added to the plates already containing growth medium to give a final concentration of 0.2 mg/ml in each well. Plates were then incubated for 3 hours. The medium containing MTT ± drug was then aspirated off and 100 µl/well isopropanol acidified with 0.04 N HCl was added. Plates were shaken for 5 minutes and absorbance was read at 570 nm on a Bio-tek EL 312e Bio-kinetics microplate reader.

Percent growth for 10001a cells was determined by dividing the absorbance reading at each drug dilution by the reading in control wells. $LD_{50}$s for each compound were determined to be the concentration of drug at which a 50% inhibition in cell growth was obtained. Potentiating effects from the combination of compounds on 10001a cells was determined by graphing the combinations of drugs which gave $LD_{50}$s in the form of an isobologram (Kallman, 1987 and Brunden, 1988).

The effectiveness of combinations of compounds with taxol on the killing of wild type, 10001a, cells is shown by means of an isobologram. The compounds of this invention act in a potentiating or synergistic manner with taxol to kill cells with much lower doses in combination than would be expected if the drugs were merely exhibiting additive effects. This effect is suprising and unexpected. The isobolograms are displayed as FIGURES 1-21. FIGURES 1-21 demonstrate the effectiveness of combinations of compounds with taxol on the killing of wild type cells. FIGURE 4 is included in the series to show how a compound with no potentiating effect behaves.

The isobologram shows the effectiveness of a combination of 2 drugs for the killing of wild type, 10001a CHO, cells. The data line is the solid line with open circle or triangle data points. The data line shows the combination of doses which gives an $LD_{50}$ for the cells. In FIGURES 1-21 the concentration of taxol is plotted against the concentration of drug. The diagonal dashed line shows the predicted concentrations of drugs if their combination only had an additive effect. If any data points were above the dashed line the date would indicate the combination of compounds had antagonistic effects. Data points below the line indicate the compounds have potentiating or synergistic effects. Compare the isobologram in FIGURE 4, showing NO potentiating effect, to the other isobolograms.

In addition to the data provided in the isobolograms the compounds have been tested in mice. Compound KT5720 has been tested on tumored mice and compounds KT5926 and

KT5720 have been tested in non-tumored mice.

Drug synergy experiments - MX-1 tumors.

FIGURE 22 shows the effect of KT5720 both separately and in combination with taxol in tumored mice. Human breast cell MX-1 tumors were implanted subcutaneously as 2 mm cubes in athymic mice. Mice were dosed every day for five days with drugs or vehicle control. The vehicle used was 2% dimethylacetamide, 10% emulphor, 88% saline. Animals received 12.5 mg/kg taxol (shown in figure as solid circle data points), 25 mg/kg KT5720 (shown in figure as open triangle data points), 12.5 mg/kg taxol + 25 mg/kg KT5720 (shown in figure as solid triangle data points), or vehicle alone (shown in figure as open square data points). Tumor burden was measured every two or three days starting with day 5 and volume was calculated. In FIGURE 22 the size of the tumor in millimeters is plotted against time in days. Eight mice were used per dose group. Results are graphed with standard errors. The results show that there was no effect of KT5720 alone on inhibition of growth of the tumor cells. Taxol, at 12.5 mg/kg, has a modest effect on reducing the tumor burden in these mice. The combination of KT5720 plus taxol clearly show a potentiation of the taxol effect by the addition of KT5720. In summary, KT5720 has no effect by itself, but in combination with taxol, at the dosage tested, it causes a dramatic inhibition of tumor growth.

Drug synergy experiments - non-tumored mice.

FIGURE 23 shows the effects of compounds KT5926 and KT5720 on non-tumored mice. When the compounds are combined with taxol and then administered to non-tumored normal mice they show a dramatic amount of toxicity. There was no lethality at the doses shown when these drugs were given individually. This means that there are strong synergistic effects with the compounds *in vivo*. The combination of drugs will be effective in tumor bearing mice and as a cancer treatment for humans. See FIGURE 23 - IN VIVO EFFECTS. This figure provides a table of data showing the toxicity of several of the drugs combined with taxol as compared to the individual administration of the drugs on non-tumored mice.

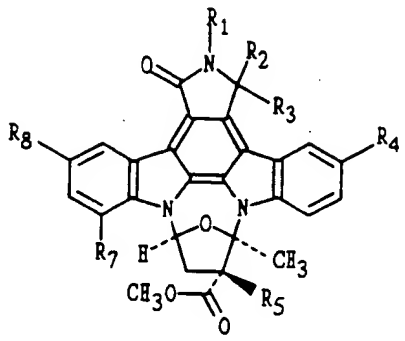
The required synergistically effective amounts (concentrations) will vary depending on the particular types of individual to be treated taking into consideration various conditions including age, weight, type of cancer treated, stage of disease, etc. Effective amounts can be readily determined by routine experimentation.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, practice the present invention to its fullest extent. The following detailed examples describe how to prepare the various compounds and/or perform the various processes of the invention and are to be construed as merely illustrative, and not limitations of the preceding disclosure in any way whatsoever. Those skilled in the art will promptly recognize appropriate variations from the procedures both as to reactants and as to reaction conditions and

techniques.

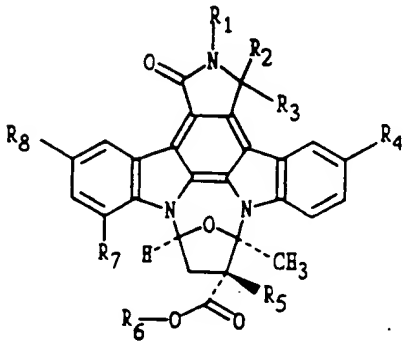
PREPARATIONS AND EXAMPLES for the preparation of novel compounds.

GENERAL REACTIONS



Starting materials

Step 1



Starting Materials. The starting materials are obtained by using the procedures described in U.S. Patent 4,923,986 and U.S. Patent 4,877,776. U.S. patents 4,923,986 and 4,877,776 are incorporated by reference into this document.

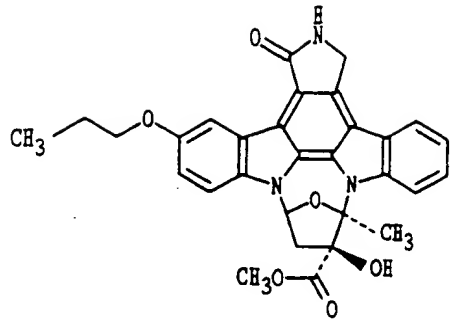
In addition to the patent references above, the starting materials for the reactions are also described in non-patent literature. The compound known as K252A is described in Kase, H., K. Iwahashi, and Y. Matsuda, K252a, "A Potent Inhibitor of Protein Kinase C from Microbial Origin." *J. Antiob. (Tokyo)* 39:10066-1071 (1986). The compound known as KT 5926, and related compounds are described in S. Nakanishi, K. Yamada, K. Iwahasha, K. Kuroda and H. Kase, "KT5926, a Potent and Selective Inhibitor of Myosin Light Chain Kinase." *Molecular Pharmocology*, 37:482-488 (1990). The other compounds of formula I where $R_6$ is $C_1$-$C_5$ alkyl are described in U.S. Patent 4,923,986 and U.S. patent 4,877,776. In general, treatment of

compounds of formula 1, where $R_6$ is $CH_3$, are treated with $R_6$-OH where ($R_6$ is $C_6$-$C_{12}$ alkyl) and KCN to give the desired compounds. All the starting materials are described in the above patents. Compounds of the type $R_5$ is H are described in WO 91/09034 published 27 June 1991. All the above documents are incorporated by reference herein.

The General Procedure for producing variations for the $R_6$ group is as follows:

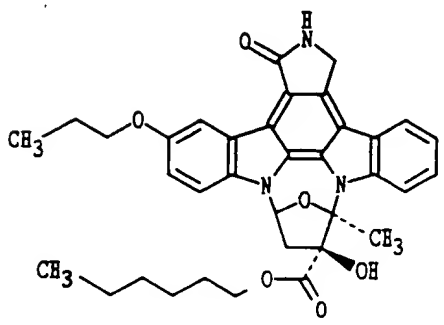
To an appropriate starting material such as KT252a add an alcohol such as n-hexanol ($R_6$ is -$(CH_2)_5CH_3$). Stir the mixture at temperatures ranging from room temperature to 125 degrees until dissolution is complete. An equal weight amount of solid KCN is added and the reaction mixture is stirred for an additional 18 to 144 hours at temperatures ranging from room temperature to 125 degrees. The reaction mixture is poured into ethyl acetate and the ethyl acetate solution is extracted with water. The organic solution is dried over anhydrous sodium sulfate, filtered and evaporated to dryness under high vacuum at temperatures ranging from 35° to 70°C to near dryness. Hexane is added to the residue and the resulting solids are allowed to sit, under hexane, for 24 hours. The solids are filtered and washed well with hexane and dried at 40°C to give the desired compound. The materials may be identified by their retention time on HPLC. Detailed HPLC conditions are provided in the examples below. For example, when the following HPLC conditions are used the retention time for KT252a is 2.54 minutes. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute).

REACTIONS OF CHART A



(KT 5926-A)

Step 1



(Example A-1)

Procedure A. Reactions of step 1, above. Preparation of **Example A-1**, ($R_8$ is -O-$(CH_2)_2CH_3$, $R_6$ is -$(CH_2)_5CH_3$ ), from the starting material KT-5926.

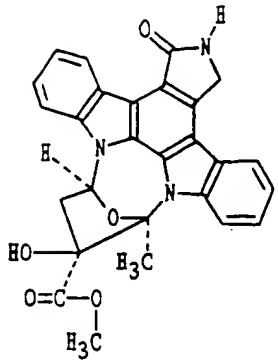
**Example A-1** is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9- methyl-1-oxo-16-propoxy-, hexyl ester, (9R-(9.alpha.,10.beta., 12.alpha.)).

To 0.8 mg of KT-5926 (0.0015 mmol) is added 0.5 ml of n-hexanol and, after dissolution is complete, 1.0 mg of KCN is added. The reaction mixture is stirred at room temperature for 24 hours at which time HPLC indicates all the starting material has reacted. Add 0.5 ml of a 35:65 water:acetonitrile solution, followed by acetonitrile (to complete dissolution) and chromatograph the entire reaction solution on a preparatory HPLC system (2-

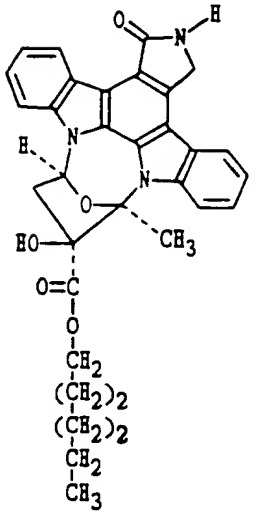
PrepPak 25 X 100 mm Cartridge) microBondapak C18, 10 microns; 35:65 water:acetonitrile at 8 ml/min taking 24 ml fractions. The fractions containing Example A-1, are combined and evaporated to dryness to give 0.12 mg of product. HPLC data was run as follows: HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) rt is 18.69 minutes for Example A-1; rt is 3.63 minutes for KT 5926.

REACTIONS OF CHART B



(K252a)

Step 1



(Example B-1)

Procedure B. Reactions of step 1, above. Preparation of Example B-1. ($R_8$ is H, $R_6$ is $-(CH_2)_6CH_3$), from the starting material, K252a.

Example B-1 is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, heptyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

To 15 mg of KT252a (0.032 mmol) add 2 ml of n-heptanol ($R_6$ is

-$(CH_2)_6CH_3$). The mixture is allowed to stir at room temperature until dissolution is complete. 15 mg of KCN is added and the reaction mixture is stirred for an additional 96 hours. The reaction mixture is poured into 20 ml of ethyl acetate and the ethyl acetate solution is extracted with water. The organic solution is dried over anhydrous sodium sulfate, filtered and evaporated to dryness under high vacuum at 59°C to near dryness. 15 ml of hexane is added to the residue and the resulting solids are allowed to sit, under hexane, for 24 hours. The solids are filtered and washed well with hexane and dried at 40°C to give 7.8 mg of Example B-1. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) retention time is 7.14 minutes for Example B-1; 2.54 minutes for K252a. Mass Spec. theory predicts 552.2498 mass units; Measured: 552.2484.

**Example B-2**, ($R_6$ is -$(CH_2)_7CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, octyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

Using procedure B only substituting n-octanol ($R_6$ is -$(CH_2)_7CH_3$) in the reaction described above, and stirring at room temperature for 120 hours, gives Example B-2, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) retention time is 3.325 minutes. Mass Spec. theory predicts 566.2655 mass units; Measured: 566.2652.

**Example B-3**, ($R_6$ is -$(CH_2)_8CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, nonyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

Using procedure B only substituting n-nonanol ($R_6$ is -$(CH_2)_8CH_3$) in the reaction described above, and stirring at room temperature for 144 hours, gives Example B-3, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (25:75 water:acetonitrile; 2 ml/minute) retention time is 6.54 minutes. Mass Spec. theory predicts 580.2811 mass units; Measured: 580.2819

**Example B-4**, ($R_6$ is -$CH(CH_2CH_3)((CH_2)_3CH_3)$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 1-ethylpentyl ester.

Using procedure B only substituting 3-heptanol ($R_6$ is -$CH(CH_2CH_3)((CH_2)_3CH_3)$ in

the reaction described above, stirring at 110 degrees for 144 hours, gives Example B-4, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) retention time is 6.44 minutes. Mass Spec. theory predicts 552.2498 mass units; Measured: 552.2501.

**Example B-5**, ($R_6$ is $-CHCH_3(CH_2)_4CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methylhexyl ester.

Using procedure B only substituting 2-heptanol ($R_6$ is $-CHCH_3(CH_2)_4CH_3$) in the reaction described above, and heating at 100 degrees for 18 hours, gives Example B-5, as an amber solid. HPLC retention time is 6.67 minutes. Mass Spec. theory predicts 552.2498 mass units; Measured: 552.2501.

**Example B-6**, ($R_6$ is $-CHCH_3(CH_2)_5CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methylheptyl ester.

Using procedure B only substituting 2-octanol ($R_6$ is $-CHCH_3(CH_2)_5CH_3$) in the reaction described above, stirring at 100 degrees for 96 hours, gives Example B-6, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (25:75 water:acetonitrile; 2 ml/minute) retention time is 4.55 minutes. Mass Spec. theory predicts 566.2655 mass units; Measured: 566.2652.

**Example B-7**, ($R_6$ is $-CHCH_3(CH_2)_6CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methyloctyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

Using procedure B only substituting 2-nonanol ($R_6$ is $-CHCH_3(CH_2)_6CH_3$) in the reaction described above, stirring at 100 degrees for 96 hours, gives Example B-7, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (25:75 water:acetonitrile; 2 ml/minute) retention time is 6.12 minutes. Mass Spec. theory predicts 580.2811 mass units; Measured: 580.2797.

**Example B-8**, ($R_6$ is $-(CH_2)_2OCH_2CH_3$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-

10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-,2-ethoxyethyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

Substituting ethoxyethanol ($R_6$ is $-(CH_2)_2OCH_2CH_3$) in the reaction described above, and stirring at room temperature for 96 hours, followed by 50 degrees for 24 hours, gives Example B-8, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) retention time is 1.54 minutes. Mass Spec. theory predicts 526.1978 mass units; Measured: 526.1959.

**Example B-9**, ($R_6$ is $-CH(cyclo\text{-}CH_2)_5$), is named:

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, cyclohexyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)).

Substituting cyclohexanol ($R_6$ is $-CH(cyclo\text{-}CH_2)_5$) in the reaction described above, stirring at 80 degrees for 18 hours, gives Example B-9, as an amber solid. HPLC: Altex Ultrasphere-ODS 0.5 micron, 4.6 mm X 25 cm, (35:65 water:acetonitrile; 2 ml/minute) retention time is 3.95 minutes. Mass Spec. theory predicts 536.2185 mass units; Measured: 536.2169.

TABLE 1 - IN VIVO EFFECTS

| | mg/kg Dose | Day of Death | |
|---|---|---|---|
| | | Mouse #1 | Mouse #2 |
| Taxol | 10.0 | *-- | -- |
| | 5.0 | -- | -- |
| KT5926 | 15.0 | -- | -- |
| | 5.0 | -- | -- |
| | 1.0 | -- | -- |
| | 0.1 | -- | -- |
| Taxol + KT5926 | 10.0 + 15.0 | 10 | 11 |
| | 10.0 + 5.0 | 7 | 10 |
| | 10.0 + 1.0 | 9 | 9 |
| | 10.0 + 0.1 | 9 | 10 |
| | 5.0 + 15.0 | 9 | 14 |
| | 5.0 + 5.0 | 9 | 10 |
| | 5.0 + 1.0 | 8 | 9 |
| | 5.0 + 0.1 | 10 | 11 |

BDF1 mice

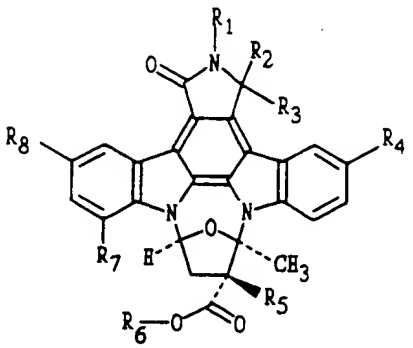
Two mice per dose (mouse #1 and mouse #2)

Drugs were given intraperitoneally on days 1-9

* No day of death given means that the mice did not die during the duration of the experiment.

## CLAIMS

1. A compound of FORMULA I, below,



FORMULA I

wherein,

$R_1$ is -H, -($C_1$-$C_4$ alkyl), -C(O)-($C_1$-$C_4$ alkyl), -$NH_2$, -C(O)-$NH_2$, -$CH_2CH_2$-N($R_{1-1}$)$_2$;

wherein $R_{1-1}$ is -H or -($C_1$-$C_4$ alkyl);

$R_2$ is -H, or $R_2$ and $R_3$ taken together are, (O);

$R_3$ is -H, -OH or $R_2$ and $R_3$ taken together are, (O);

$R_4$ is -H, -OH, -$NH_2$, or -O-($C_1$-$C_4$ alkyl);

$R_5$ is -OH, -O-($C_1$-$C_4$ alkyl), or -O-C(O)-($C_1$-$C_4$ alkyl);

$R_6$ is -($C_6$-$C_{12}$ alkyl), -($C_3$-$C_{10}$ cycloalkyl),($C_1$-$C_5$ alkyl)-O-($C_1$-$C_5$alkyl), -$(CH_2)_n$$CH_2$N($R_{6-1}$)$_2$;

wherein $R_{6-1}$ is -H, or -($C_1$-$C_4$ alkyl);

$R_7$ is -H, or -$NH_2$;

$R_8$ is -Cl, -Br, -H, -$CH_3$, -$CH_2$OH, -OH, -O-($C_1$-$C_4$ alkyl); -N($R_{8-1}$)$_2$, or -NHC(O)-N($R_{8-1}$)$_2$;

wherein $R_{8-1}$ is -H or -($C_1$-$C_4$ alkyl);

wherein n is 0-5

with the proviso that:

a) when $R_2$ or $R_3$ is -OH then $R_1$ is H;

b) when $R_1$, $R_2$, $R_3$, $R_4$, and $R_7$ all equal H and $R_5$ is OH then $R_6$ does NOT equal -$(CH_2)_5CH_3$;

c) when $R_1$, $R_4$, and $R_7$ all equal H, and $R_2$ combined with $R_3$ is (O) and $R_5$ is OH , then $R_6$ does NOT equal -$(CH_2)_5CH_3$;

d) when $R_4$ is -OH, -$NH_2$, or -O-($C_1$-$C_4$ alkyl), then $R_4$ and $R_8$ are the same.

2. A compound of claim 1 wherein
$R_1$ is -H, -($C_1$-$C_4$ alkyl), -C(O)-($C_1$-$C_4$ alkyl), -C(O)-$NH_2$.

3. A compound of claim 1 wherein
$R_1$ is H or -$CH_3$.

4. A compound of claim 1 wherein
$R_1$ is -$CH_2CH_2$-N($R_{1-1}$)$_2$.

5. A compound of claim 1 wherein
$R_2$ and $R_3$ are H.

6. A compound of claim 1 wherein
$R_5$ is -OH.

7. A compound of claim 1 wherein
$R_5$ is -$OCH_3$.

8. A compound of claim 1 wherein
$R_6$ is -($C_7$-$C_{12}$ alkyl), -($C_3$-$C_{10}$ cycloalkyl), -$(CH_2)_n CH_2$N($R_{6-1}$)$_2$.

9. A compound of claim 1 wherein
$R_6$ is -($C_8$-$C_{12}$ alkyl), -($C_3$-$C_{10}$ cycloalkyl), -$(CH_2)_n CH_2$N($R_{6-1}$)$_2$.

10. A compound of claim 2 wherein
$R_2$ and $R_3$ is (O).

11. A compound of claim 2 wherein
$R_1$ is -H, -($C_1$-$C_4$ alkyl);
$R_2$ and $R_3$ are H and
$R_5$ is -OH or -O-($C_1$-$C_4$ alkyl).

12. A compound of claim 5 wherein
$R_5$ is -OH or -O-($C_1$-$C_4$ alkyl).

13. A compound of claim 5 wherein

$R_8$ is -Cl, -Br, -H, $-CH_3$, $-CH_2OH$, -OH, $-O-(C_1-C_4$ alkyl), $-N(R_{8-1})_2$, or $-NHC(O)-NHR_{8-1}$.

14. A compound of claim 5 wherein

$R_8$ is $-O-(C_1-C_4$ alkyl).

15. A compound of claim 11 wherein

$R_1$ is -H;

$R_8$ is $-O-(C_1-C_4$ alkyl).

16. A compound of claim 15 which is the compound named,

9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9- methyl-1-oxo-16-propoxy-, hexyl ester, (9R-(9.alpha.,10.beta., 12.alpha.)). (Example A-1)

17. A compound of claim 11 selected from the following named compounds,

a) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, heptyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)), (Example B-1)

b) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-,1-ethylpentyl ester, (Example B-4) or

c) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methylhexyl ester (Example B-5).

18. A compound of claim 11 wherein

$R_1$ is -H;

$R_6$ is $-(C_8-C_{12}$ alkyl) and

$R_8$ is $-O-(C_1-C_4$ alkyl).

19. A compound of claim 11 wherein

$R_1$ is -H;

$R_6$ is -($C_3$-$C_{10}$ cycloalkyl) or ($C_1$-$C_5$ alkyl)-O-($C_1$-$C_5$ alkyl) and

$R_8$ is -O-($C_1$-$C_4$ alkyl).

20. A compound of claim 11 wherein

$R_1$ is -H;

$R_6$ is -($C_8$-$C_{12}$ alkyl) and

$R_8$ is H.

21. A compound of claim 20 selected from the following named compounds.

a) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, octyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)); (Example B-2)

b) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, nonyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)); (Example B-3)

c) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methylheptyl ester; (Example B-6)

d) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, 2-methyloctyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)) (Example B-7).

22. A compound of claim 11 wherein

$R_1$ is -H;

$R_6$ is -($C_3$-$C_{10}$ cycloalkyl) or ($C_1$-$C_5$ alkyl)-O-($C_1$-$C_5$ alkyl) and

$R_8$ is H.

23. A compound of claim 22 selected from.

a) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4- i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-,2-ethoxyethyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)); (Example B-8) or

b) 9,12-Epoxy-1H-diindolo(1,2,3-fg:3',2',1'-kl)pyrrolo(3,4-i)(1,6)benzodiazocine-10-carboxylic acid, 2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-, cyclohexyl ester, (9R-(9.alpha.,10.beta.,12.alpha.)) (Example B-9).

24. A pharmaceutical composition for treating, controlling or preventing cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia in mammals and humans, which comprises administering a therapeutic or prophylactic dosage of a compound of FORMULA I in conjunction with an appropriate dose of taxol or taxol related compounds.

25. A pharmaceutical composition for treating, controlling or preventing cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia, in mammals and humans, which comprises administering a therapeutic or prophylactic dosage of any one of the compounds selected from the list below, in conjunction with an appropriate dose of taxol or taxol related compounds, a) KT5823, b) K252a; c) KT5926, d) KT5720, e) Staurosporine, f) driamycin, b) Amilorides, c) Calphostin, d) Chlorpromazine, e) The compound known as "HA-1004", f) Indomethacin, g) Okadaic acid, h) Phenazocine, i) Polymyxin, j) 2-aminopurine k) 6-dimethyl-aminopurine, l) Sphingosine, m) Tamoxifen, n) Compounds related to tamoxifen such as triphenylethylene antiestrogens, o) Trifluoperazine, p) Verapamil, q) 3-isobutyl-1-methyl-xanthine, r) 8-Cl-cAMP, in conjunction with an appropriate dose of taxol or taxol related compounds.

26. Use of a therapeutic or prophylactic dosage of a compound of FORMULA I in conjunction with an appropriate dose of taxol or taxol related compounds for the manufacture of a medicament for the treatment, control or prevention of cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia in mammals and humans.

27. Use of a therapeutic or prophylactic dosage of the compounds described in U.S. Patents 4,877,776 or 4,923,986, in conjunction with an appropriate dose of taxol or taxol related compounds for the manufacture of a medicament for the treatment, control or prevention of cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia in mammals and humans.

28. Use of a therapeutic or prophylactic dosage of any of the compounds selected from the following: a) KT5823, b) K252a, c) KT5926, d) KT5720, or e) Staurosporine, in conjunction with an appropriate dose of taxol or taxol related compound for the manufacture of a medicament for the treatment, control or prevention of cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia in mammals and humans.

29. Use of a therapeutic or prophylactic dosage of any of the compounds selected from the following:

a) Adriamycin
b) Amilorides
c) Calphostin
d) Chlorpromazine
e) The compound known as "HA-1004"
f) Indomethacin
g) Okadaic acid
h) Phenazocine
i) Polymyxin B
j) 2-aminopurine
k) 6-dimethyl-aminopurine
l) Sphingosine
m) Tamoxifen
n) Compounds related to tamoxifen such as triphenylethylene antiestrogens
o) Trifluoperazine
p) Verapamil
q) 3-isobutyl-1-methyl-xanthine or
r) 8-Cl-cAMP

in conjunction with an appropriate dose of taxol or taxol related compound for the manufacture of a medicament for the treatment, control or prevention of cancerous growths, such as human ovarian tumors, mammary tumors, and malignant melanoma, lung tumors, gastric tumors, colon tumors, head and neck tumors, and leukemia in mammals and humans.

FIGURE 1



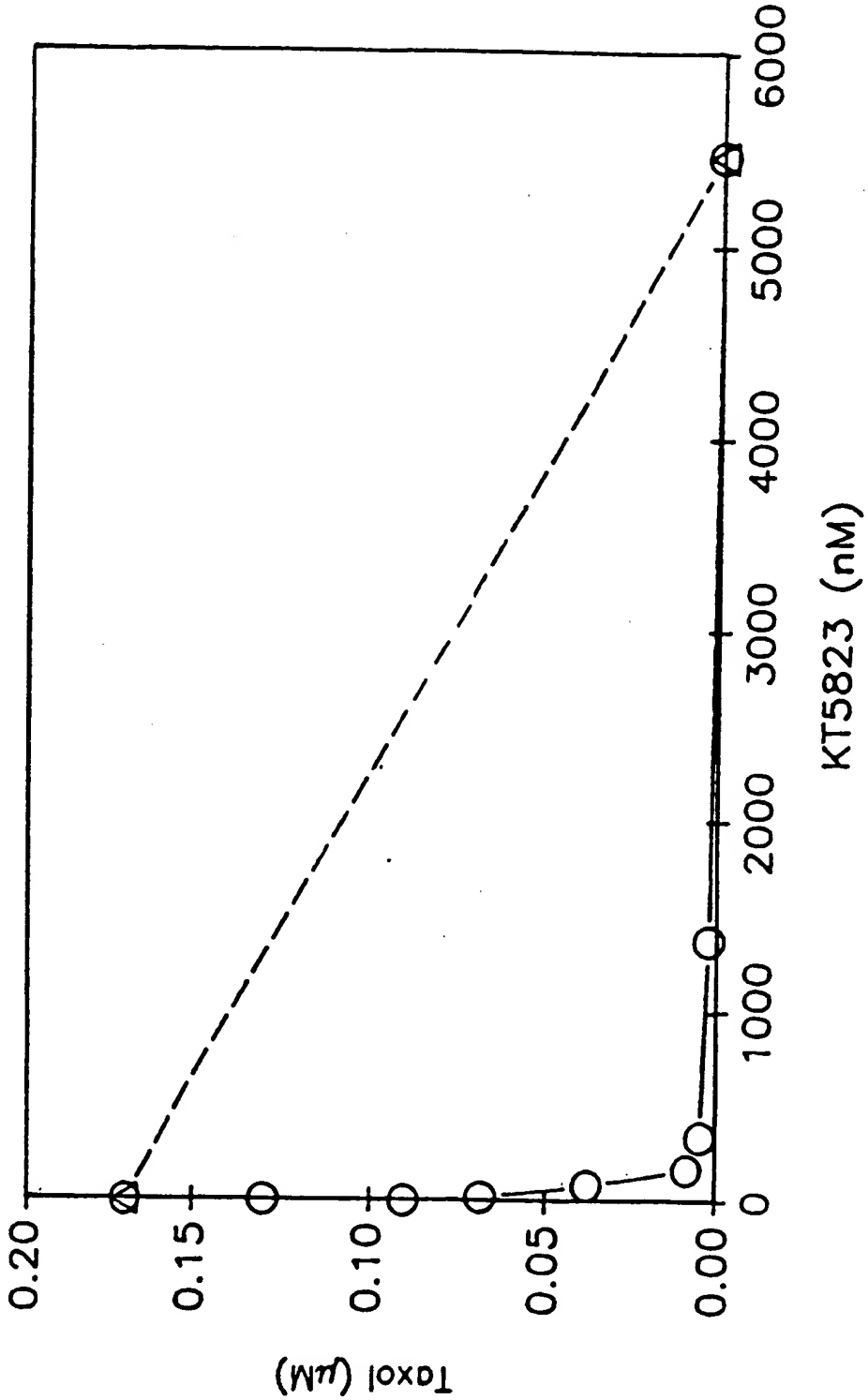



FIGURE 1.

FIGURE 2 2/22

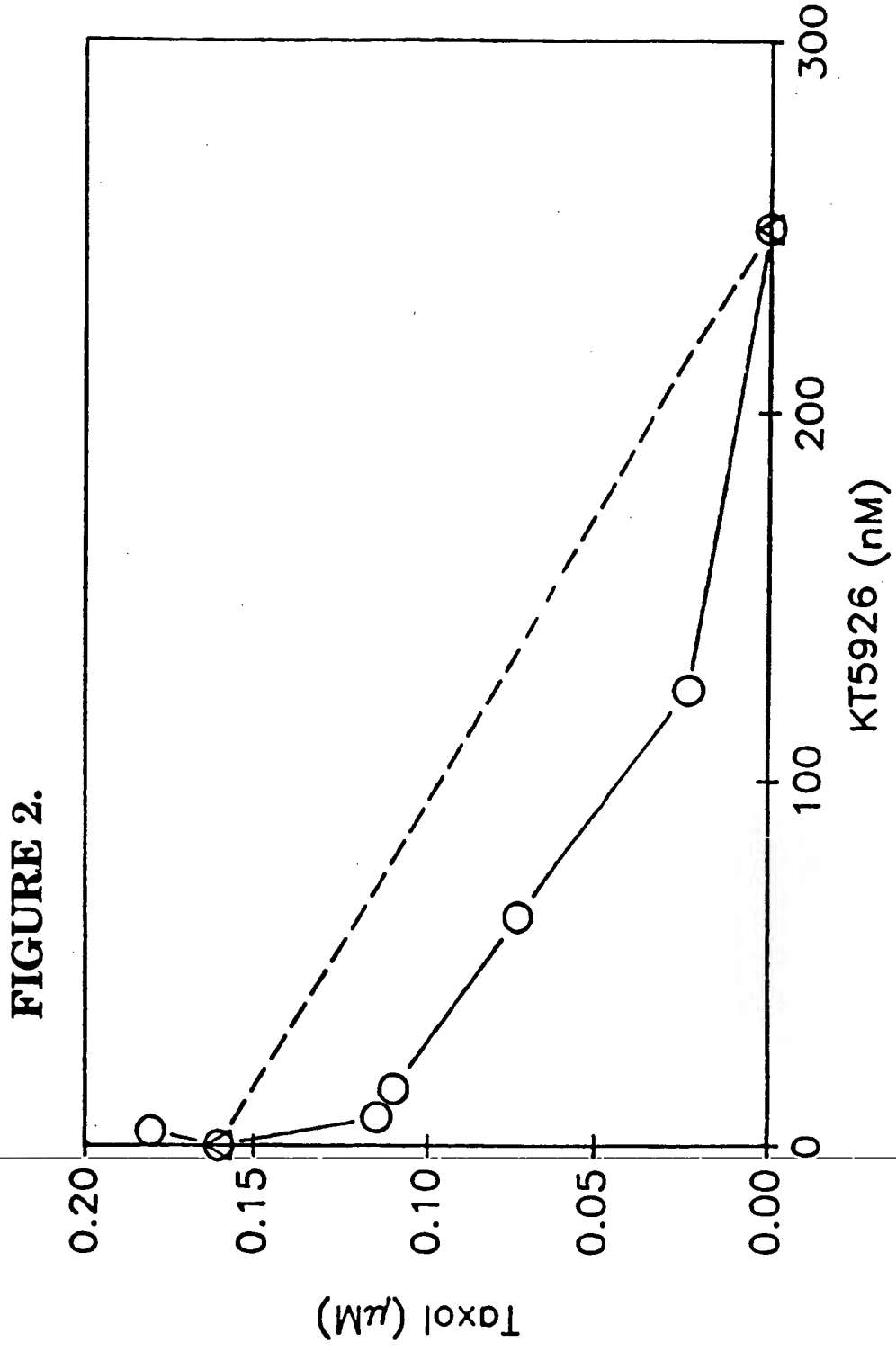



FIGURE 2.

FIGURE 3



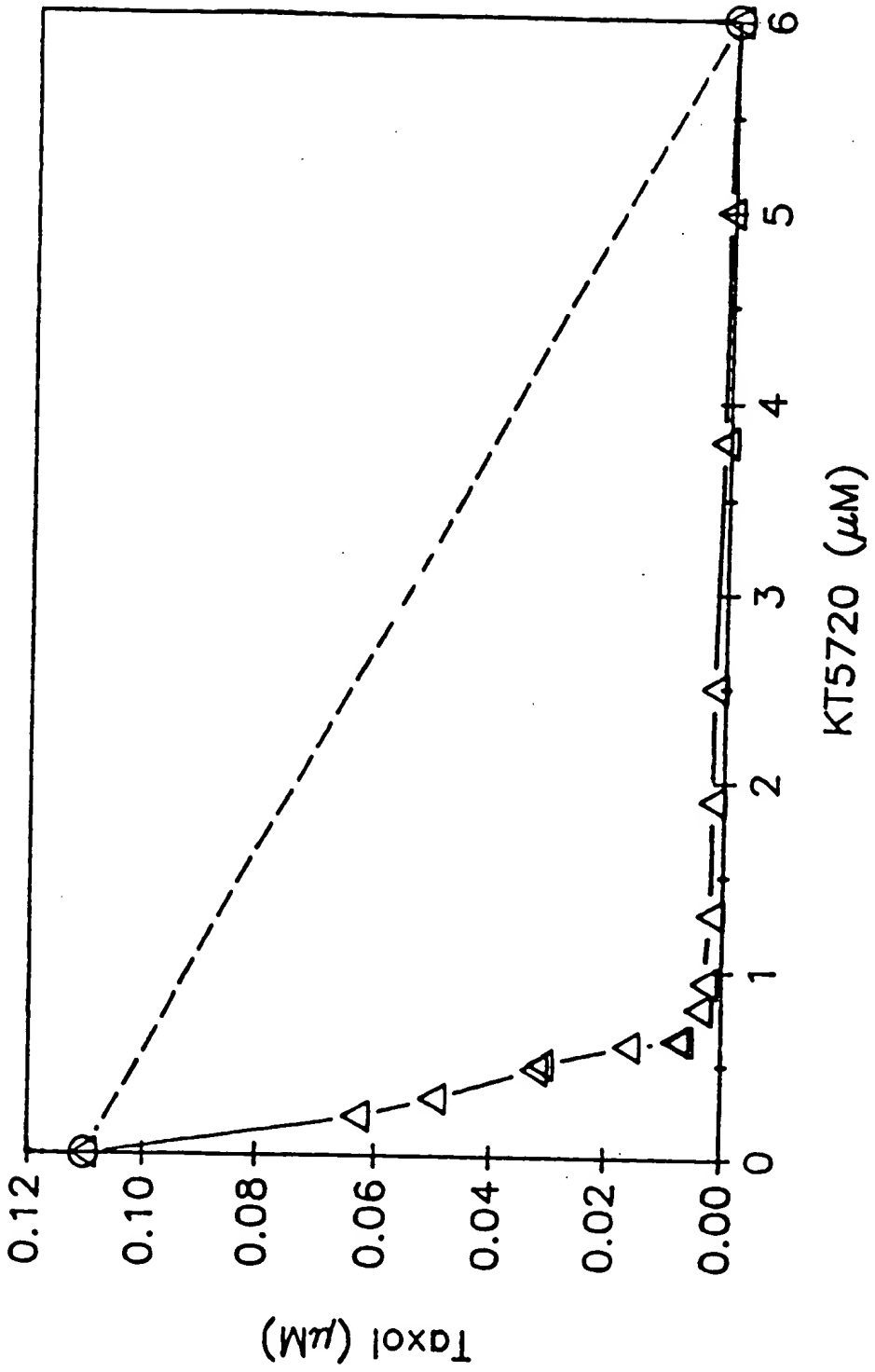



**FIGURE 3.**

FIGURE 4 4/22

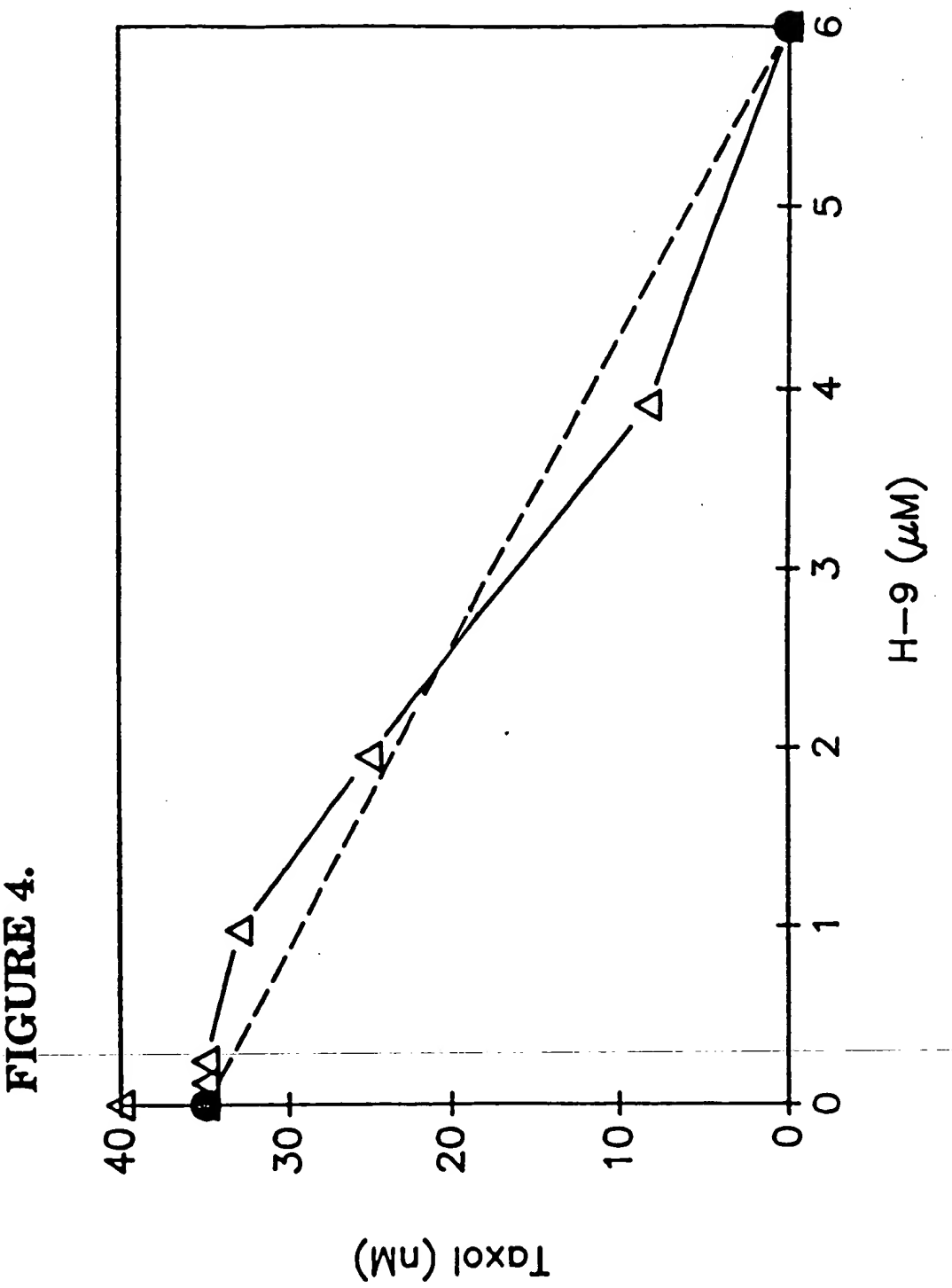



FIGURE 4.

FIGURE 5 5/22

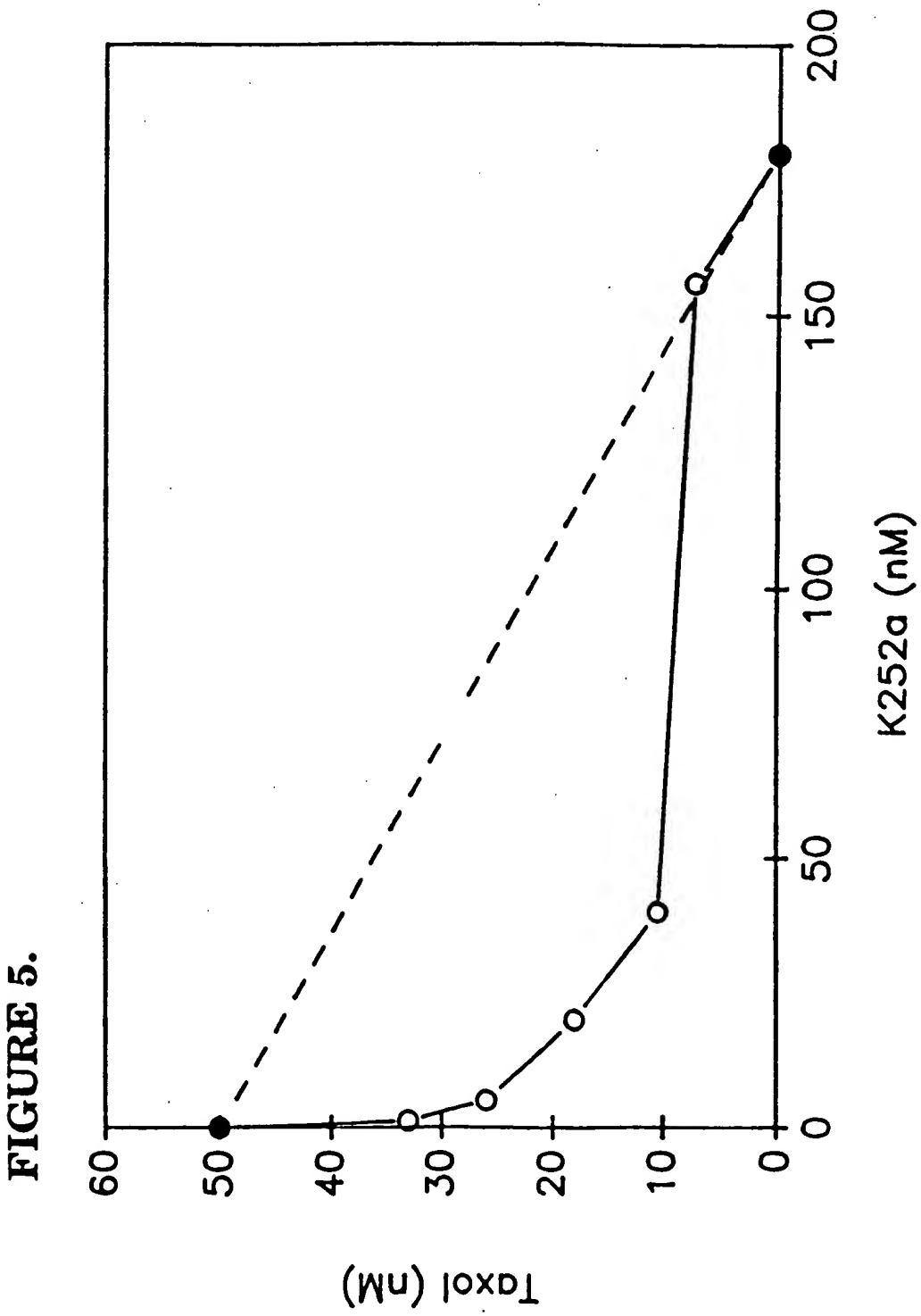



FIGURE 5.

FIGURE 6 6/22

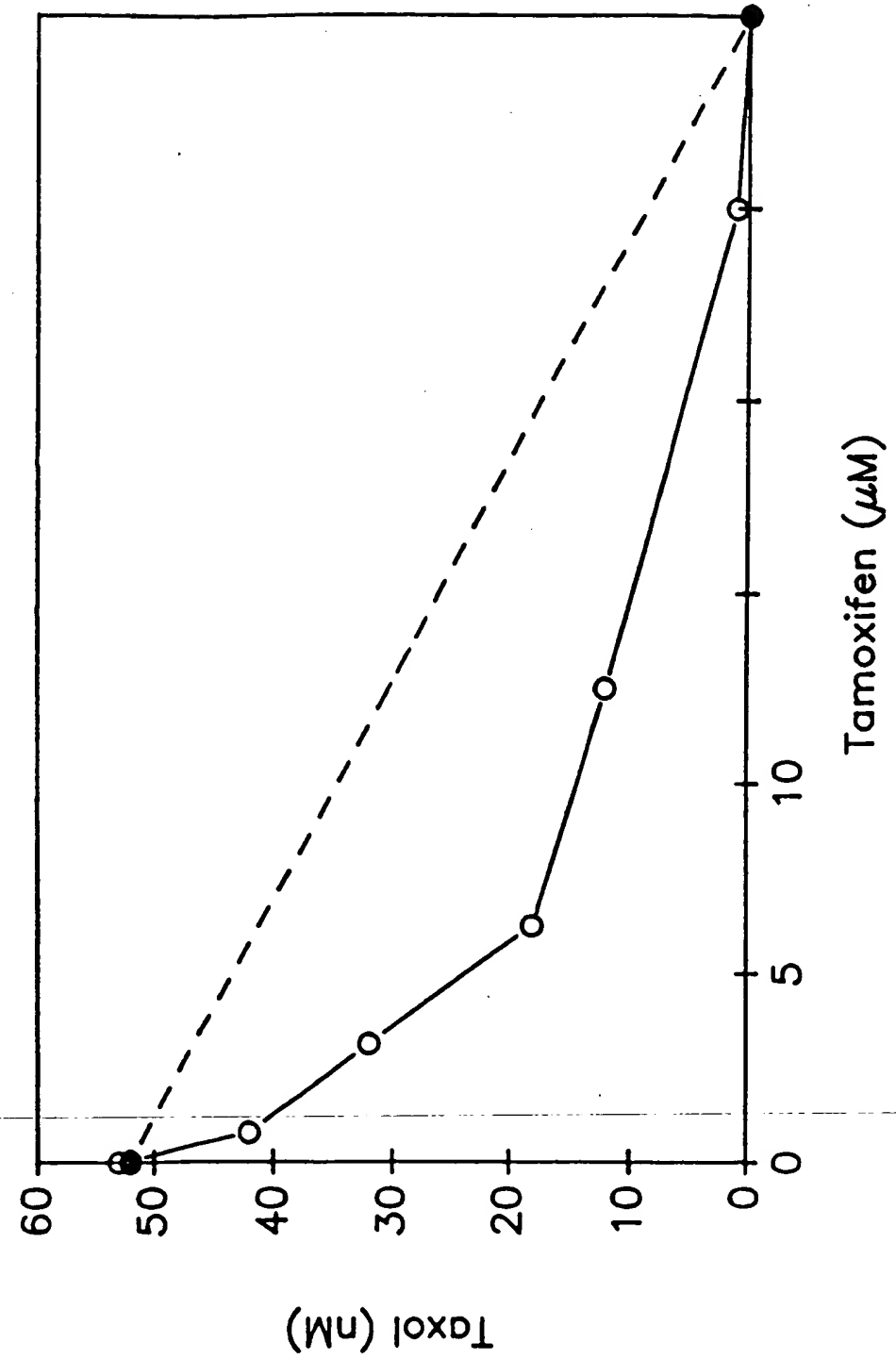



FIGURE 6.

FIGURE 7 7/22

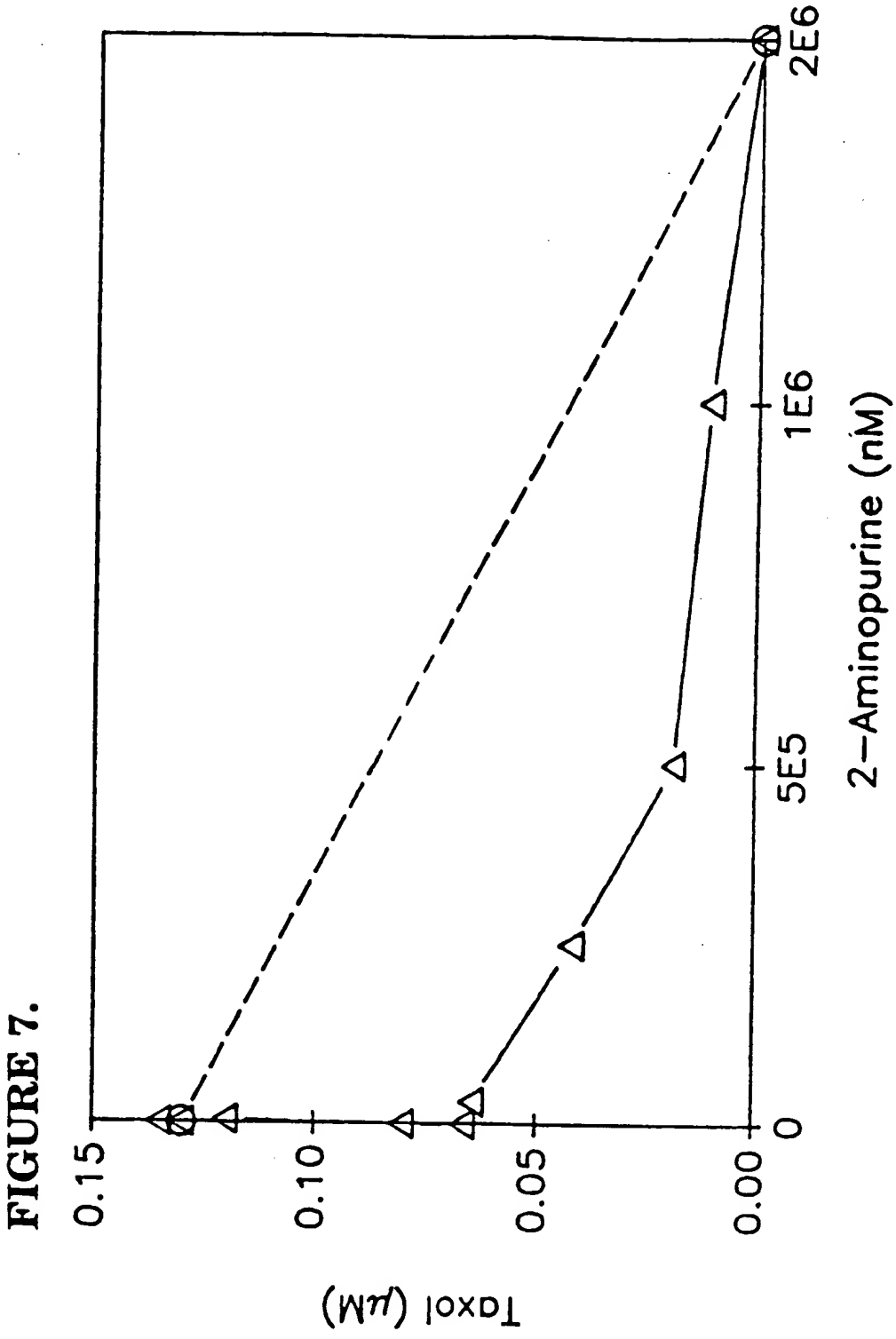



FIGURE 7.

FIGURE 8 8/22

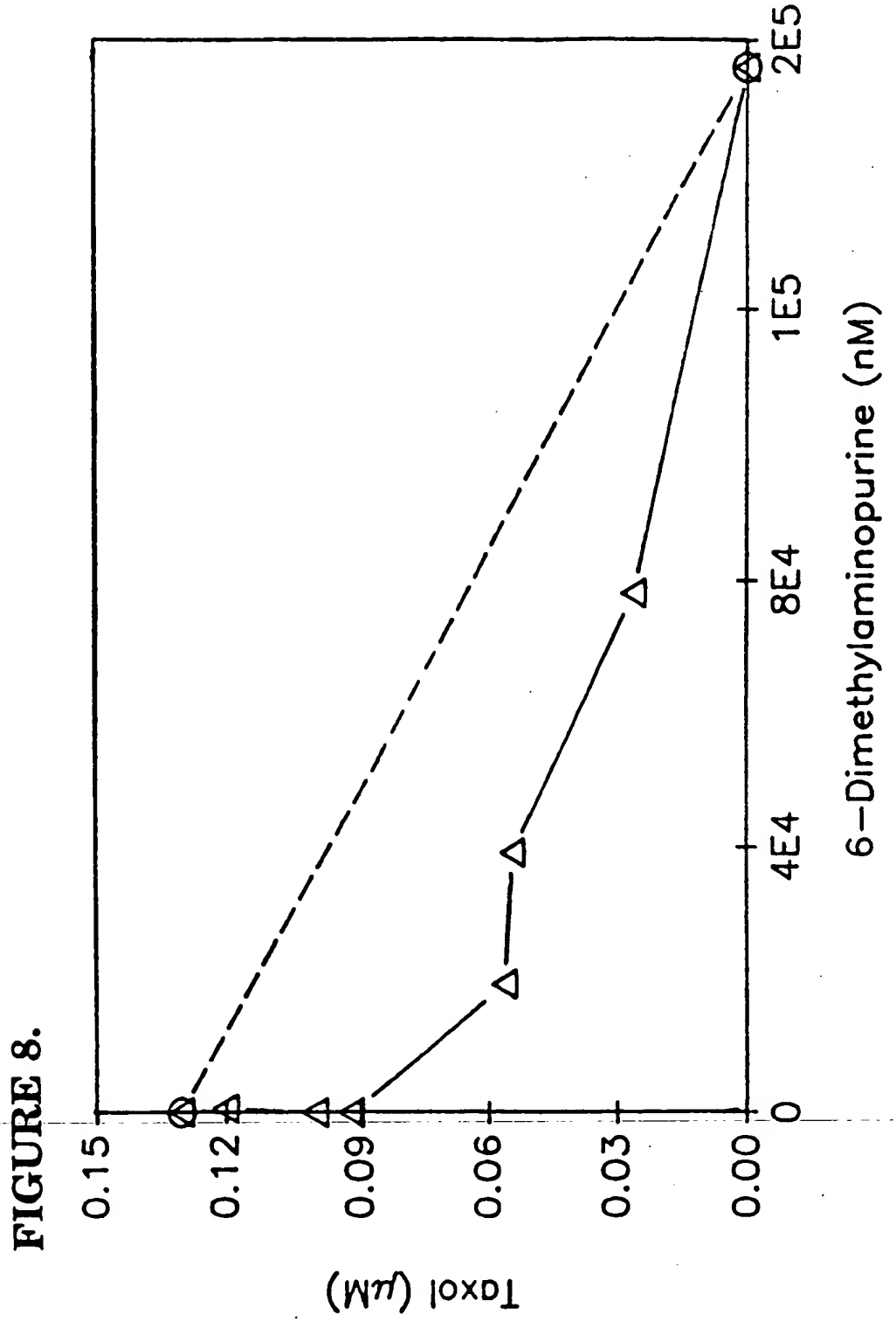



FIGURE 8.

FIGURE 9 9/22

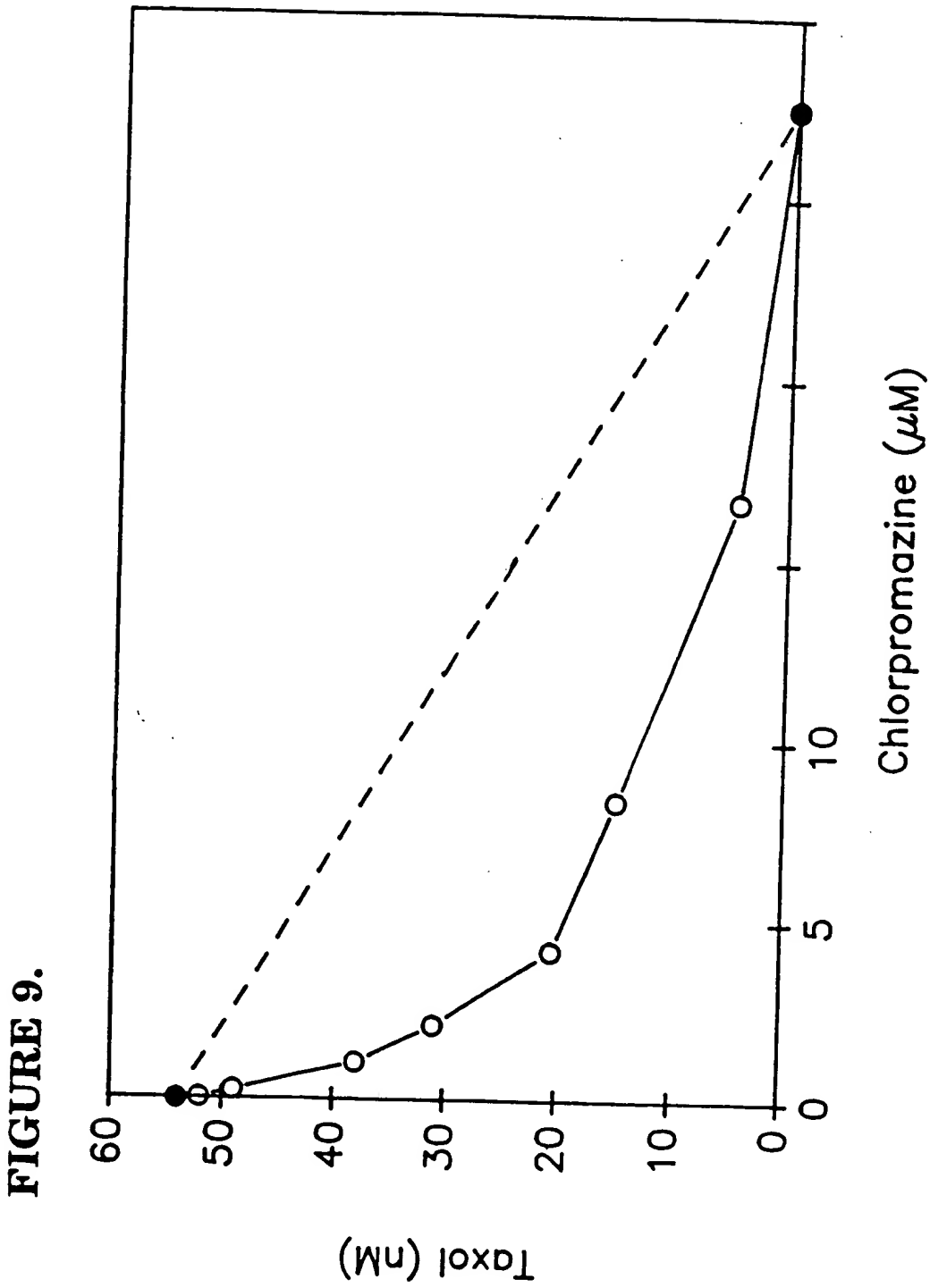



FIGURE 9.

FIGURE 10 10/22

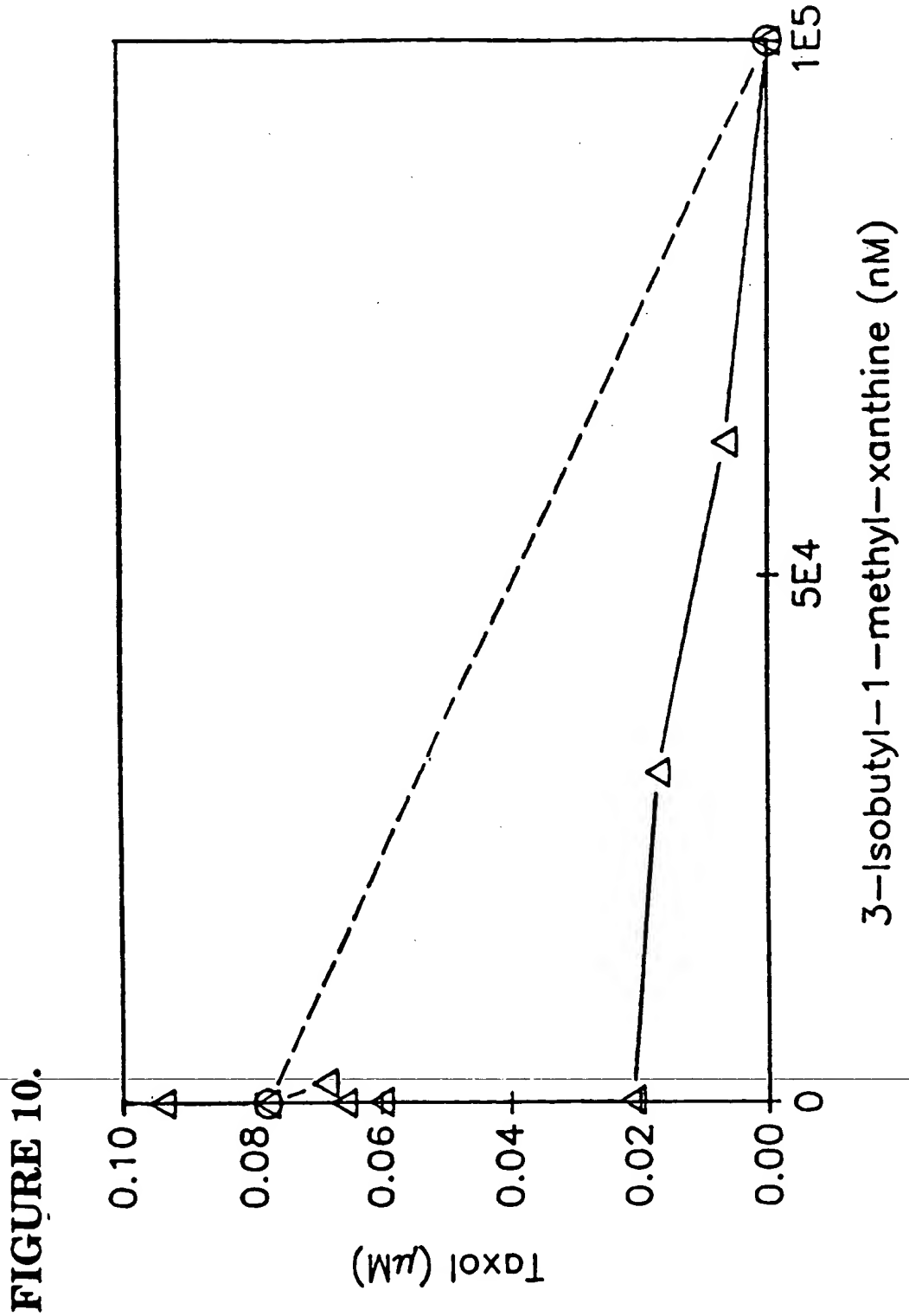



FIGURE 10.

FIGURE 11 11/22

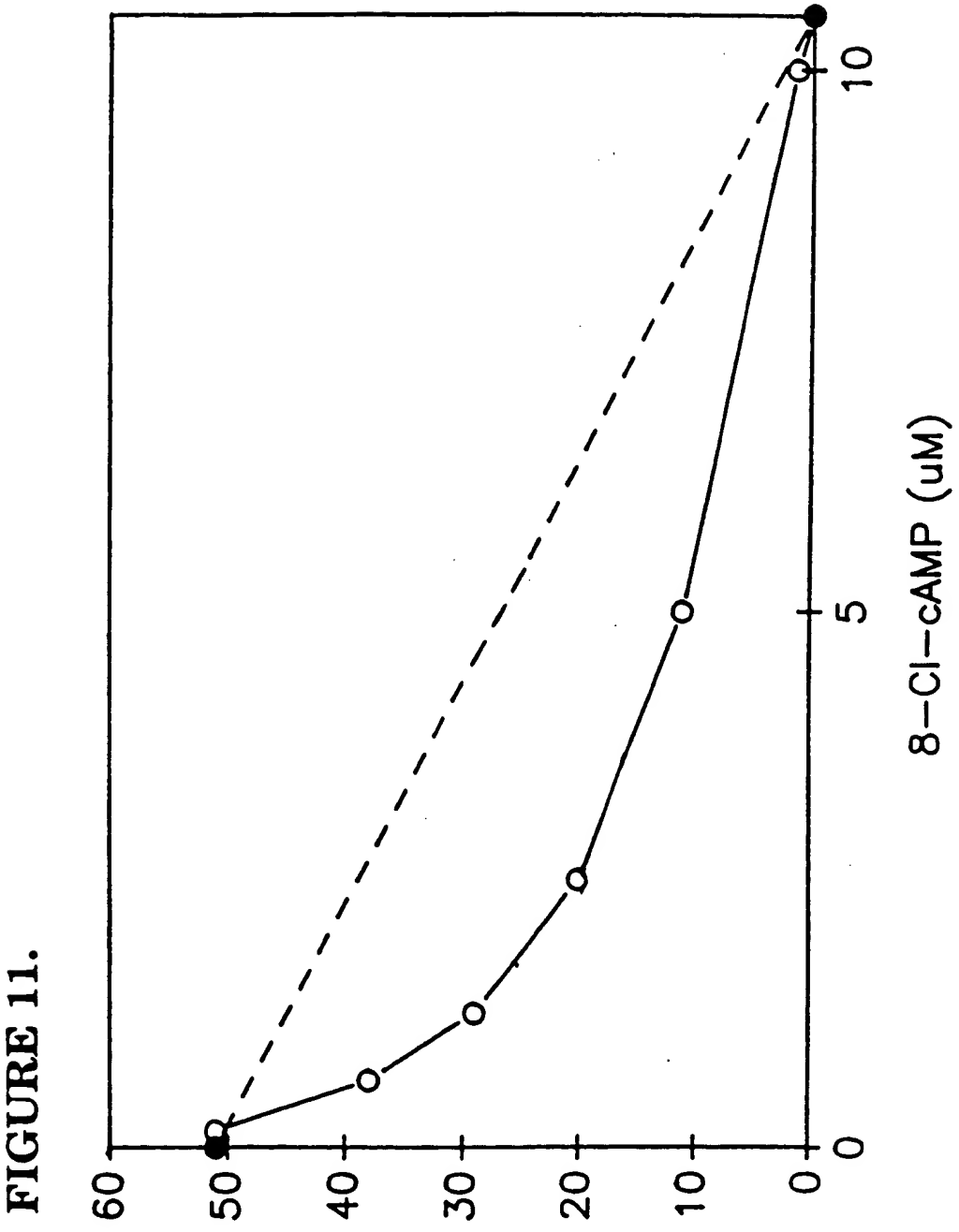



FIGURE 11.

FIGURE 12 12/22

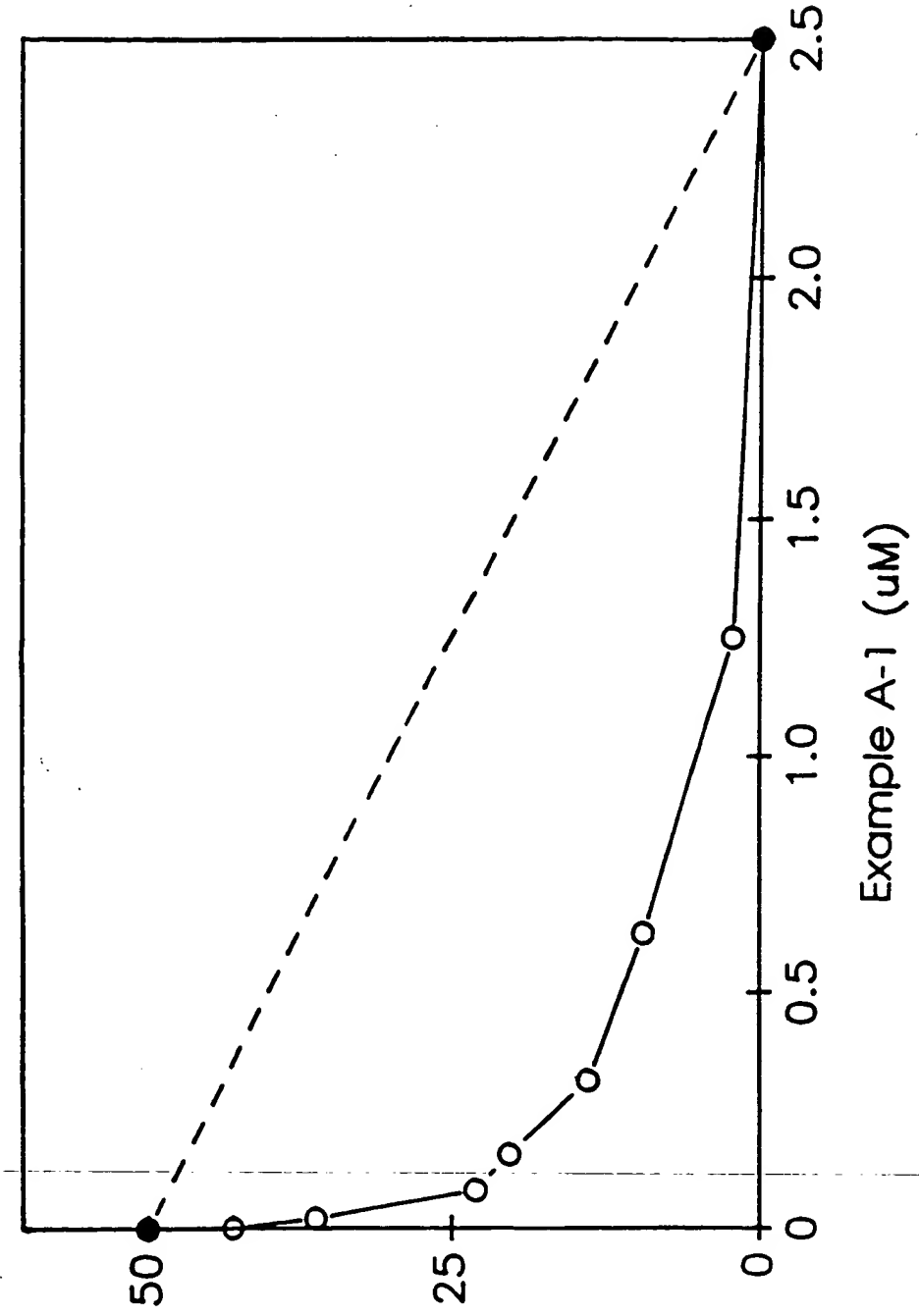



FIGURE 12.

FIGURE 13 13/22

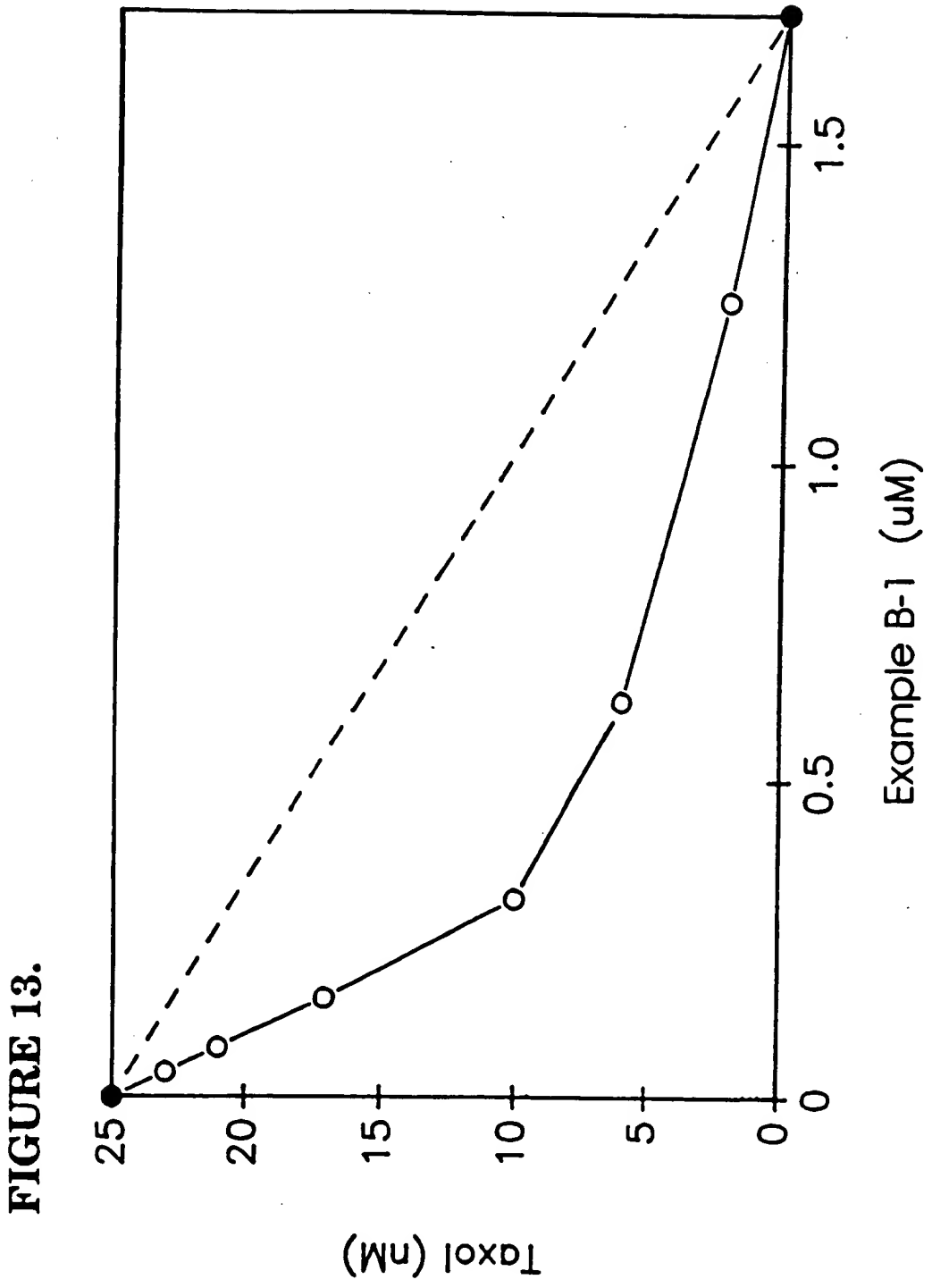



FIGURE 13.

FIGURE 14 14/22

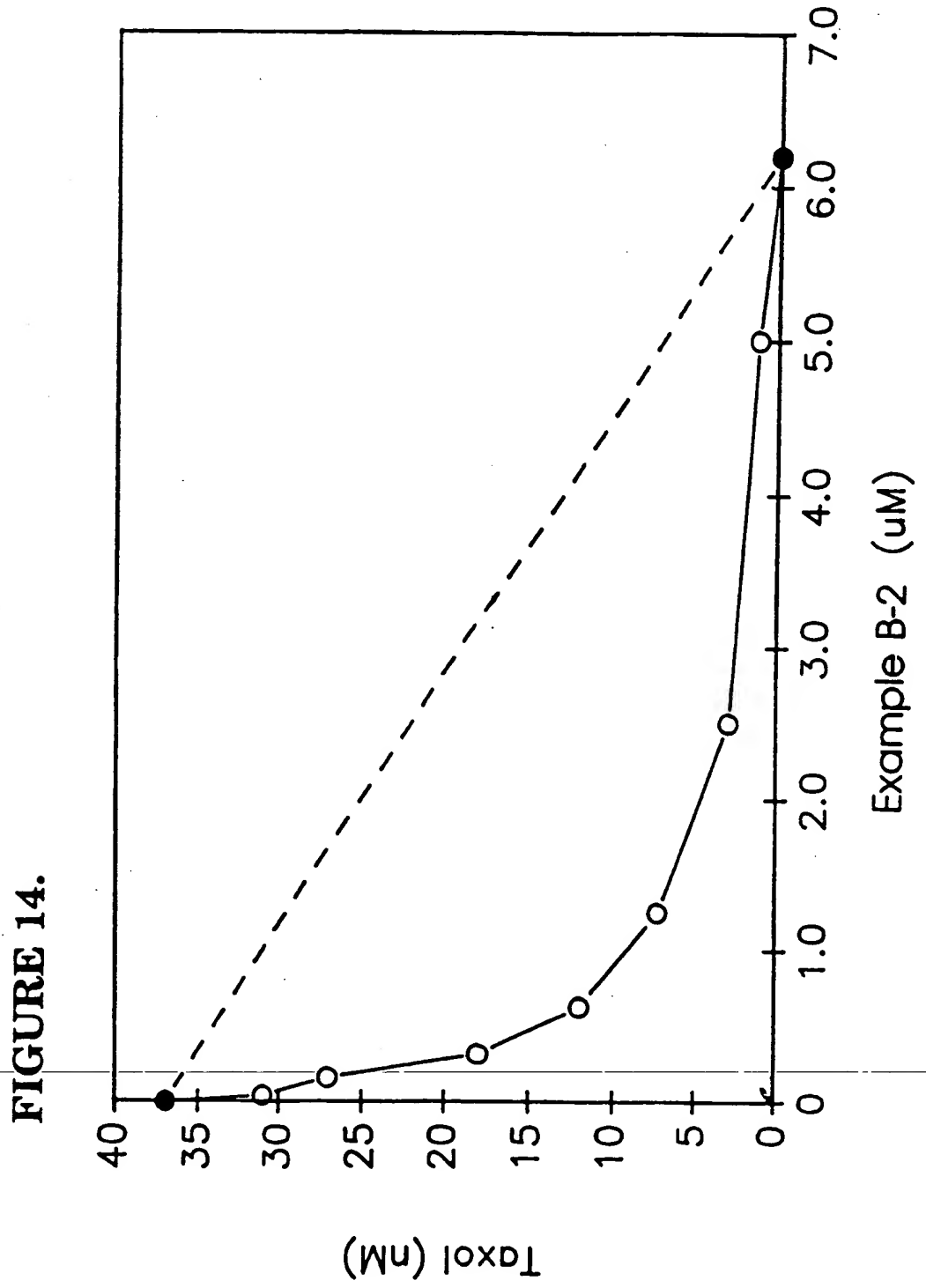



FIGURE 14.

FIGURE 15 15/22

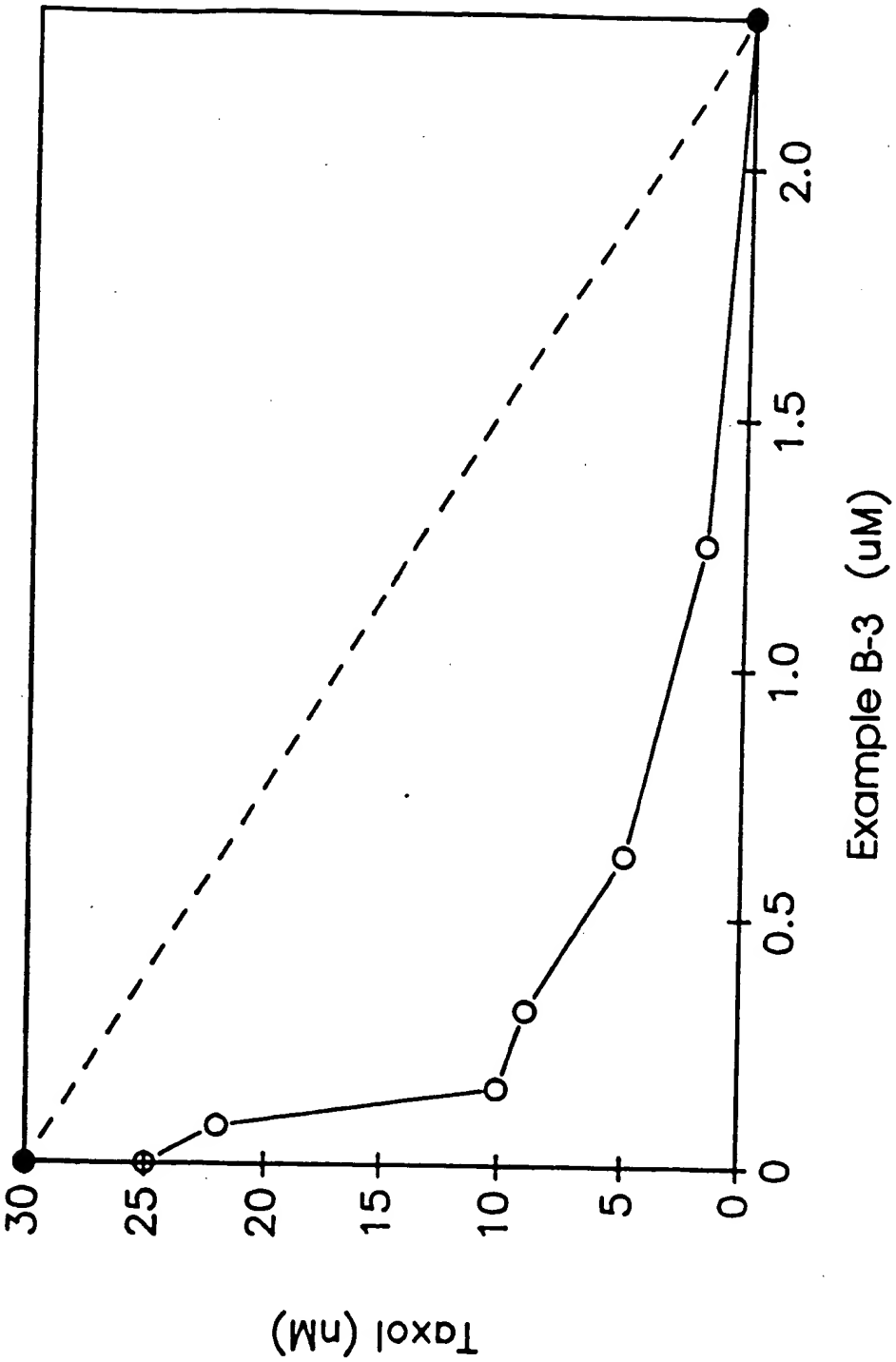



FIGURE 15.

FIGURE 16 16/22

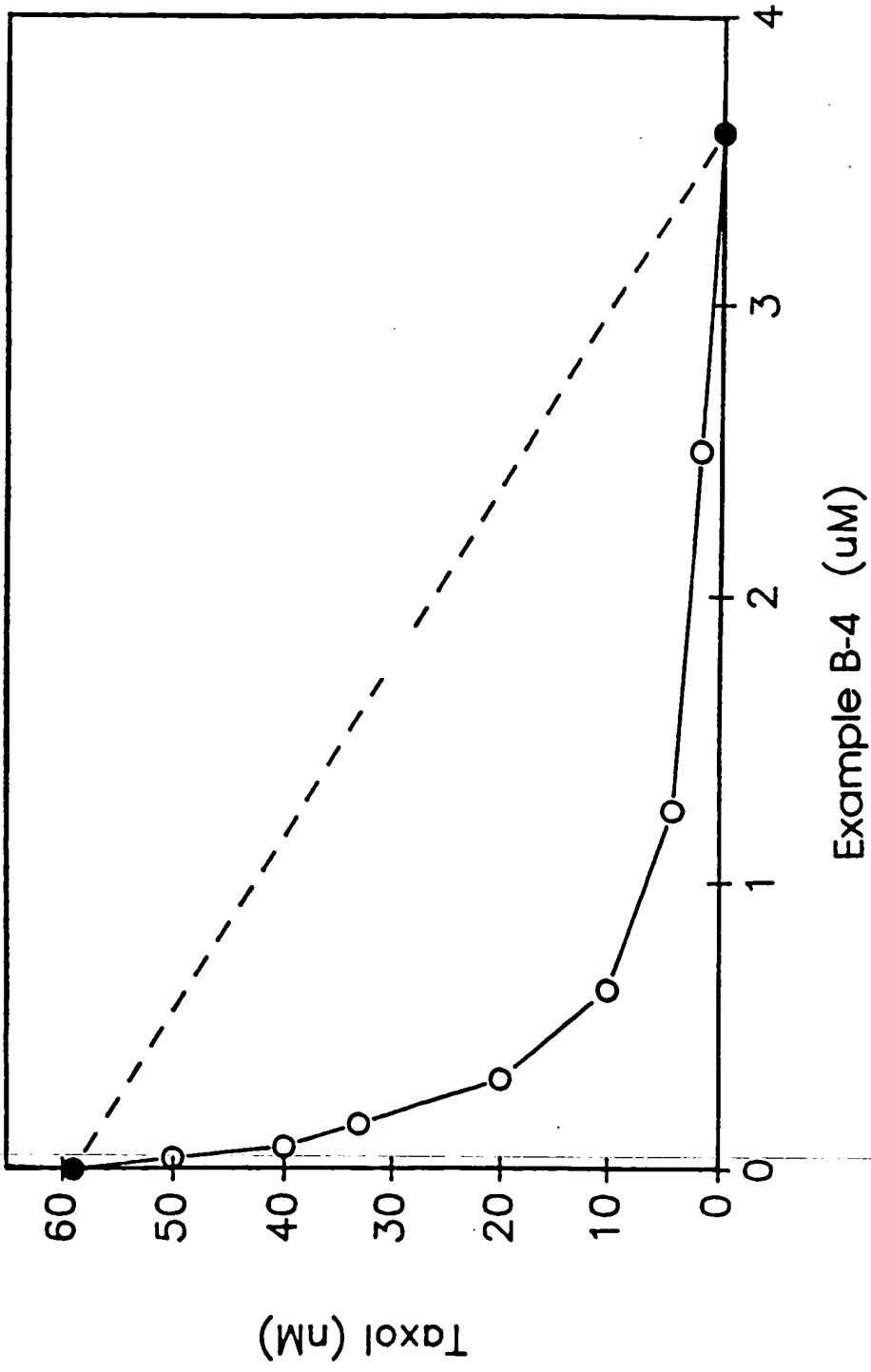



FIGURE 16.

FIGURE 17 17/22

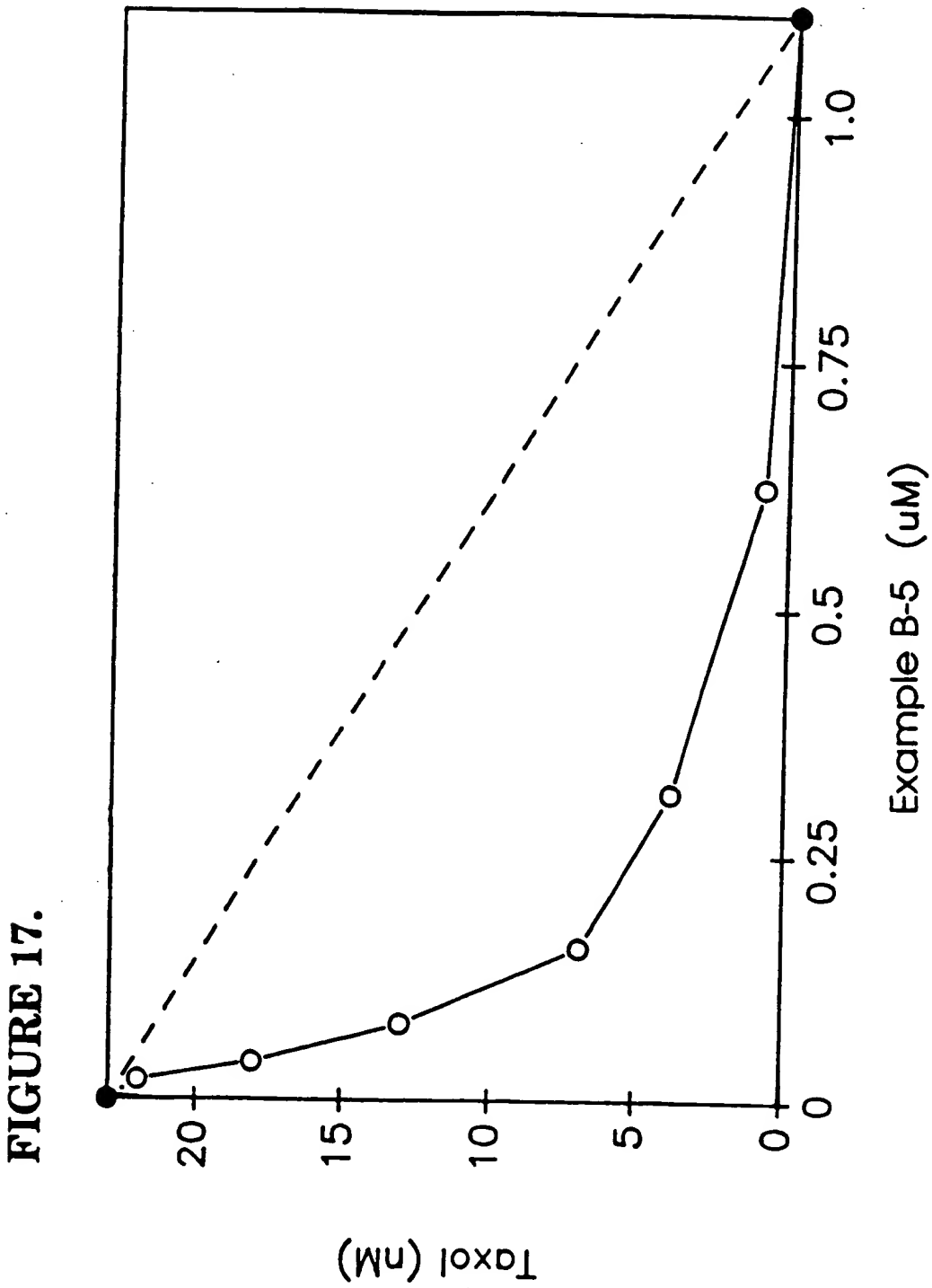



FIGURE 17.

FIGURE 18 18/22

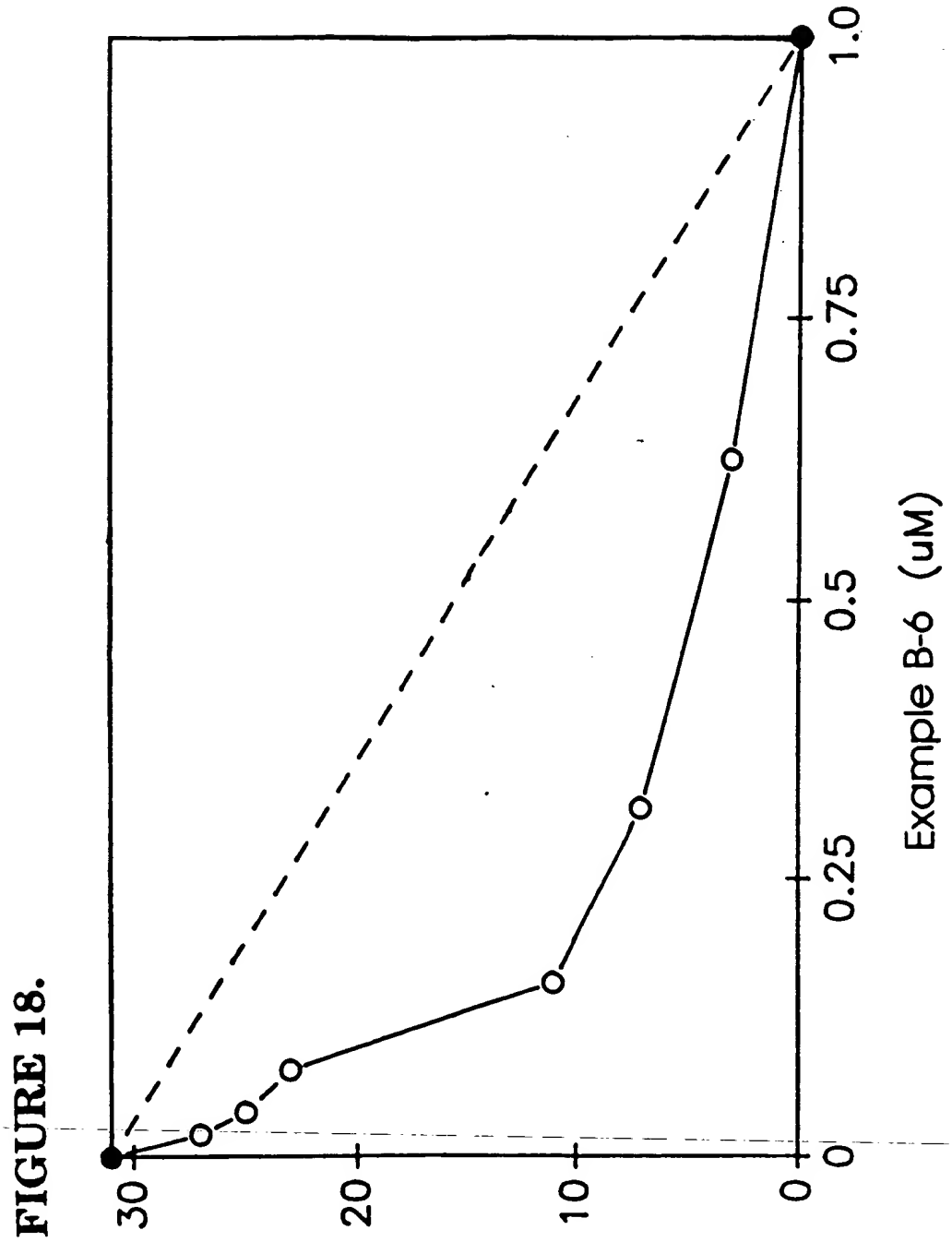



FIGURE 18.

FIGURE 19 19/22

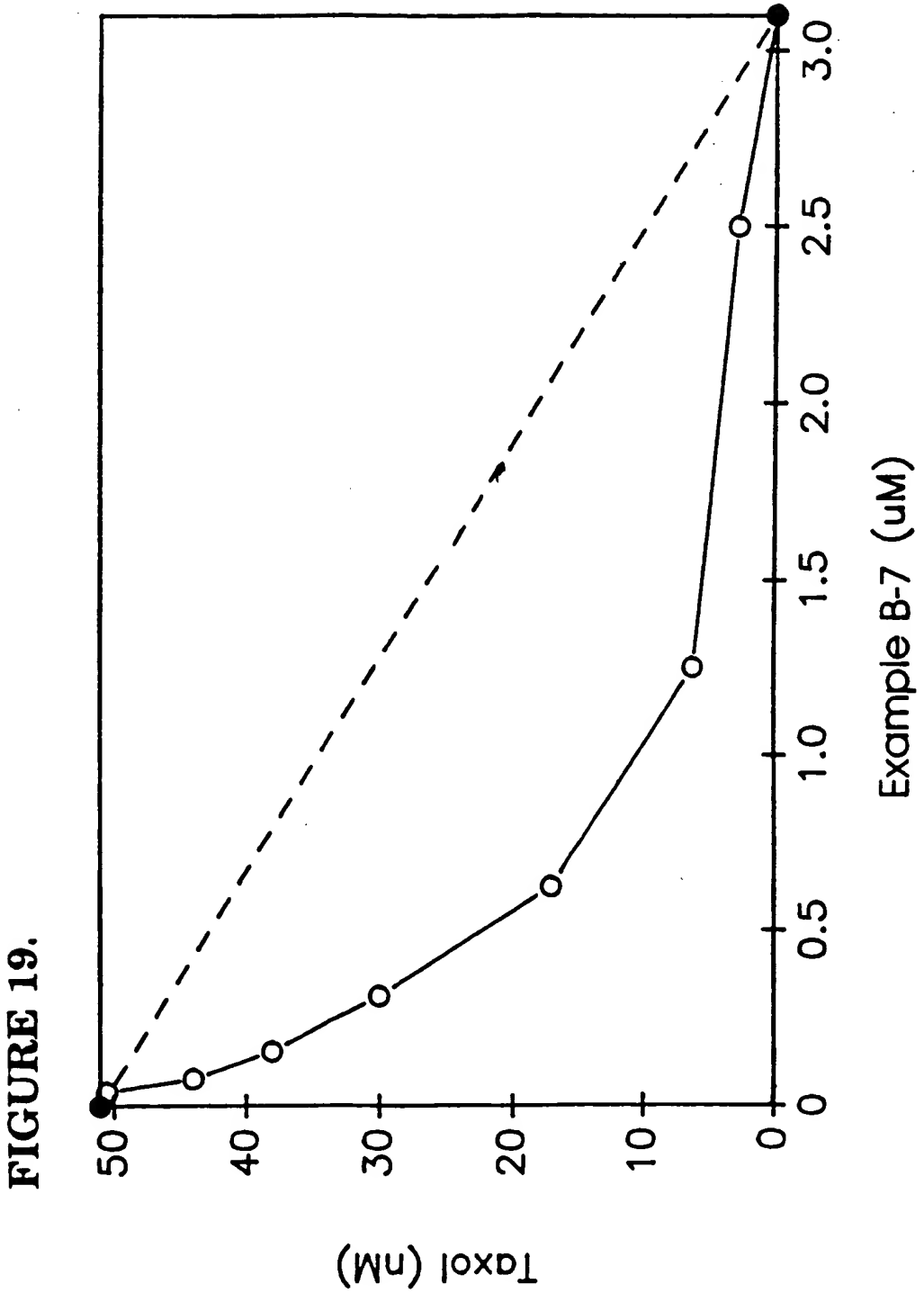



FIGURE 19.

FIGURE 20 20/22

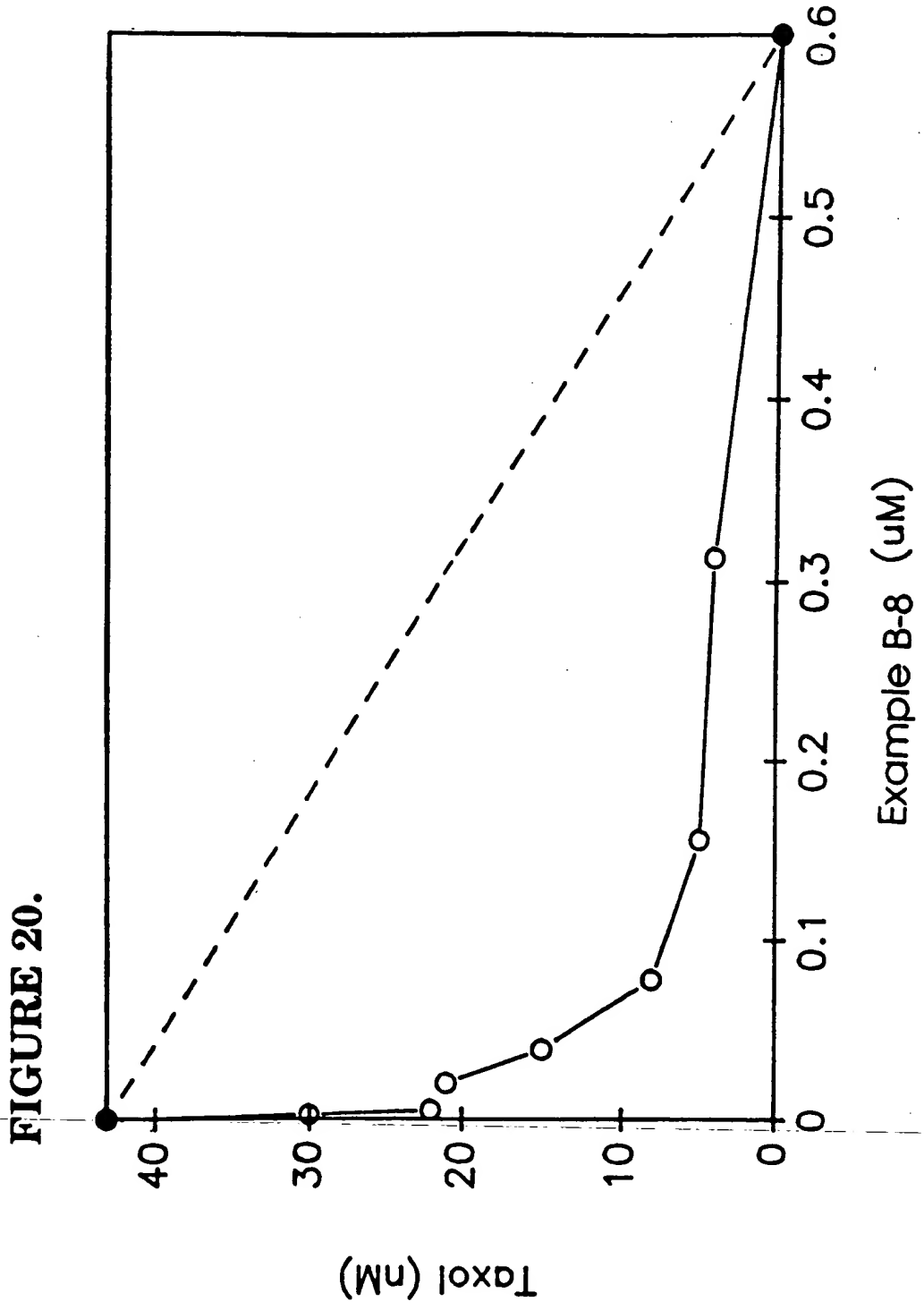



FIGURE 20.

FIGURE 21



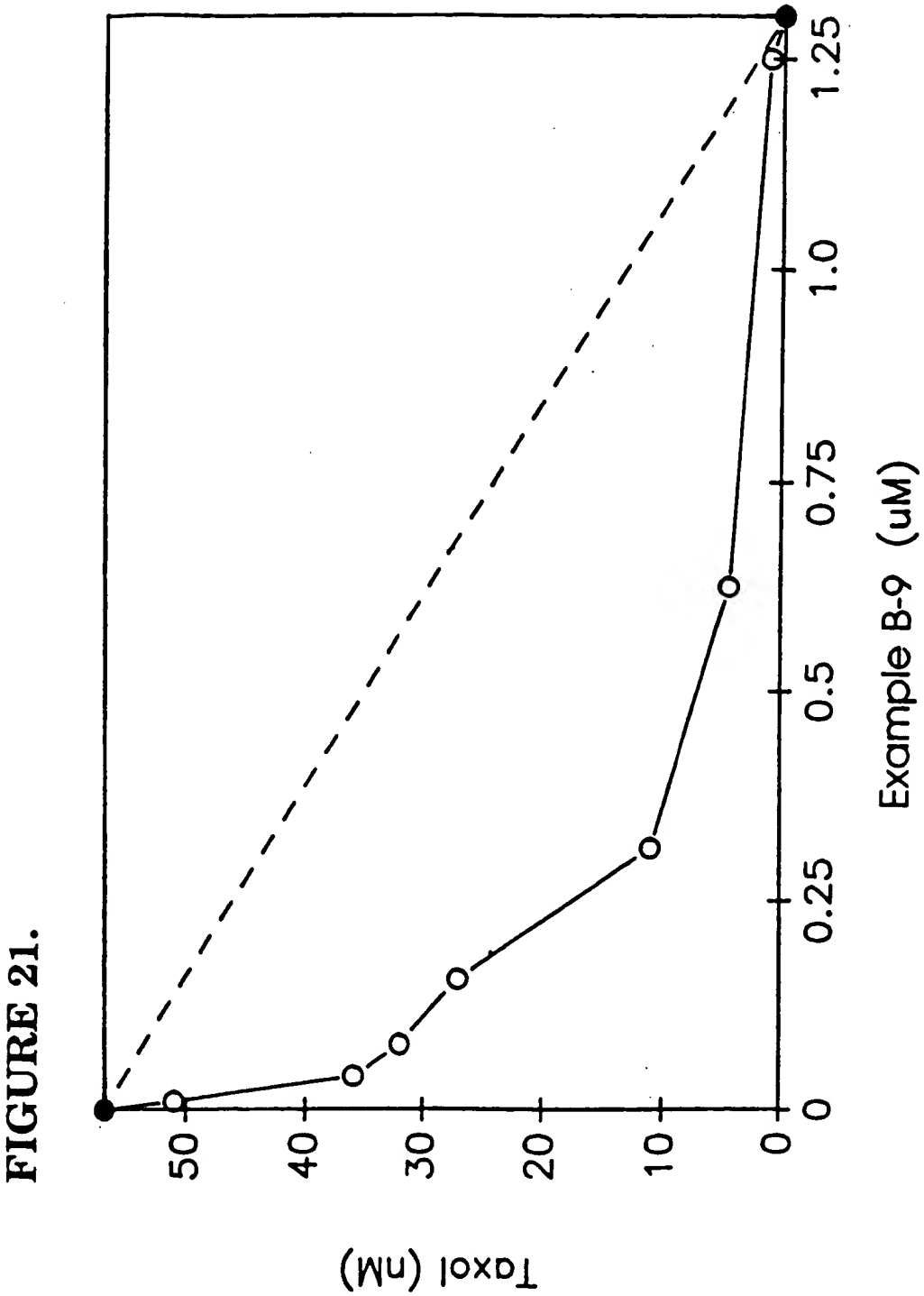



FIGURE 21.

FIGURE 22 22/22

# FIGURE 22. TAXOL PLUS KT5720 ON TUMORED MICE